The

Structure and Function of

MUSCLE

Volume III

Volume I Structure

Volume II Biochemistry and Physiology

Volume III Pharmacology and Disease

The Structure and Function of MUSCLE

Edited by

G H BOURNE

Department of Anatomy
Emory University
Atlanta, Georgia

Volume III

PHARMACOLOGY AND DISEASE



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PREFACE

The first two volumes have dealt with the structure and function of muscle. The first part of this volume relates to the effects of drugs on all three types of muscle and the subsequent chapters deal with abnormal muscle. Specific infections, certain nutritional deficiencies, old age and death all inflict a pattern of alteration on muscular structure, all affect muscular function to a spectacular degree in the last condition. Intrinsic diseases of muscle cause changes which are reflected clinically and these diseases seem to follow a pattern of inheritance which is discussed in this volume.

We are most happy to conclude this volume and this whole treatise with some general comments on muscle by such a distinguished authority as Albert Szent-Györgyi. His studies and his writings on muscle over the last twenty years have sparked interest, controversy and research all over the world and we feel that his dynamic approach to muscle structure and function is the right note on which to bring our story to a close.

This third volume will have special interest for pharmacologists, pathologists, neurologists, and those clinicians who are interested in neuromuscular diseases, to gerontologists and nutritionists.

GEOFFREY H BOURNE

Department of Anatomy
Emory University
Atlanta, Georgia
June 1960

CONTRIBUTORS TO VOLUME III

E. B. BECKETT Department of Histology University of Liverpool, England

J. R. BENDALL, Low Temperature Research Station, Cambridge, England.

F. D. BOSANQUET West End Hospital for Neurology and Neurosurgery and Maida Vale Hospital for Nervous Diseases, London, England.

G. H. BOURNE, Department of Anatomy Emory University Atlanta, Georgia.

MARION deV. COTTEN, Division of Basic Health Sciences, Emory University Atlanta, Georgia.

P. M. DANIEL, Department of Neuropathology Institute of Psychiatry The Maudsley Hospital, London, England

E. J. FIELD Department of Pathology Royal Victoria Infirmary Newcastle-upon Tyne, England.

P. C. C. GARNHAM, Department of Parasitology and Helminthology, London School of Hygiene and Tropical Medicine, London, England.

RONALD A. HENSON Department of Medicine, London Hospital Medical College, London, England and Department of Medicine, Institute of Neurology, London, England.

KARL E. MASON Department of Anatomy School of Medicine and Dentistry Rochester New York.

NEIL C. MORAN Division of Basic Health Sciences, Emory University, Atlanta, Georgia.

H. B. PARRY Nuffield Institute for Medical Research, University of Oxford England.

L. J. RUBINSTEIN Department of Psychiatry and Neurology University of Minnesota, The Medical School, Minneapolis, Minnesota.

ALBERT SZENT-GYÖRGYI, Institute for Muscle Research, Marine Biological Laboratory Woods Hole, Massachusetts.

STEPHEN THESLEFF University of Lund Sweden.

JOHN N. WALTON Neurological Unit, Royal Victoria Infirmary Newcastle-upon-Tyne, England.

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across the surface membrane of a single muscle cell, and to obtain faithful records of its changes during activity In particular this technique has advanced our knowledge of neuromuscular transmission and consequently has also furthered our understanding of drug effects on these processes (cf. Castillo and Katz, 1956) In this chapter, the section devoted to striated muscle is based mainly on recent pharmacological studies with the aforementioned technique, and on such problems that have arisen as a result of this work.

A complete survey of the response of smooth muscle to the multitude of drugs which act upon it is beyond the scope of this presentation. Only a brief orientation can be given of some general problems of drug action and of the effects of various pharmacologically important drugs. In my opinion, however this limitation is not serious, since two recent and comprehensive reviews on the pharmacology of smooth muscle are available. One deals with the effects of drugs on intestinal muscle (Vaughan Williams, 1954) and the other with the pharmacology of vascular smooth muscle (Furchgott, 1955)

II. DRUGS AFFECTING SMOOTH MUSCLE

Drugs may affect smooth muscle by a stimulatory or inhibitory effect on structures remote from the muscle tissue, and/or by local action on the smooth muscle cell. The former remotely initiated type of drug action, comprises effects on for instance, structures concerned with the nervous and humoral control of muscle cells. Although this mode of action is of great pharmacological importance the remote site of the effect excludes it from this presentation. Here, only drugs whose site of attack is spatially within the region of tissue containing smooth muscle will be discussed

Local action of drugs on smooth muscle may be of two types (Furchgott, 1955) One is denoted as "local indirect action," and results from reaction of a drug with some component of the muscle cell or of adjacent tissues, leading to the release or accumulation of a second substance which acts on the muscle The second type is called "direct action" and results from reaction of a drug with certain specific components (receptors) of the smooth muscle cell This is a schematic classification and, as will be seen, a drug action is in many instances a combination of both types.

The effects of drugs on the electrical properties of the smooth muscle cell will be considered separately

A. Local Indirect Action

A frequently occurring mode of local indirect action is when a drug stimulates peripheral autonomic ganglia, thereby causing release of the postganglionic transmitter agent. Depending upon the nature of the chemical transmitter and upon the type of muscle tissue, this produces either stimulation or inhibition of the contractile elements in the smooth muscle cell. Drugs like nicotine acetylcholine, barium, and histamine probably act partly in this way on intestinal smooth muscle. However all these agents also affect the smooth muscle directly by acting on specific drug receptors on the cell surface. Although attempts have been made by several workers to separate and compare the local indirect and the direct action of these drugs, no conclusive results have hitherto been obtained. As a generalization, it may be said that since these agents have both stimulatory and inhibitory effects on autonomic ganglia, as well as on the smooth muscle cell the extent to which the mechanical response of the muscle tissue is modified by the respective drug is complex. Consequently the predominant site of action of these drugs tends to vary with the experimental conditions. In view of such difficulties, it is not surprising that the results obtained in various laboratories have been conflicting, and that interpretation of the main site of action of the aforementioned drugs on intestinal muscle is still controversial (for a review see Vaughan Williams, 1954)

A typical example of local indirect action is provided by the effects of anticholinesterase drugs. The main pharmacological effect of these agents in moderate concentrations is an inactivation of enzymes responsible for the destruction of acetylcholine. Cholinesterase inhibitors allow acetylcholine to accumulate at the effector cell, and their action on smooth muscle is therefore similar to that of externally applied acetylcholine. When added in high concentrations to isolated smooth muscle preparations, these drugs may affect the muscle cell directly but it is questionable whether this occurs with the concentrations used *in vivo* (Koelle and Gilman, 1949)

A similar mode of action has been proposed for certain sympathomimetic amines. Ephedrine, tyramine, and related compounds have been found to inhibit monoamine oxidase, an enzyme which is probably responsible to some extent for inactivation of the sympathetic transmitters in the body This observation, and the finding that these drugs affect smooth muscle only in the presence of adrenergic agents, have

led a number of investigators to suggest that their sympathomimetic activity is due mainly to a local accumulation of the sympathetic transmitter caused by inactivation of the monoamine oxidase (Gaddum and Kwiatkowski, 1938, Burn 1953) The correctness of this hypothesis has, however been questioned by several authors (see reviews by Bacq 1949 Blaschko 1952, Furchgott, 1955)

Epinephrine is generally believed to exert its excitatory and inhibitory effects on smooth muscle by a direct action. Recently however a theory has been put forward that the inhibitory action of epinephrine is due to "local indirect action" (Mohme Lundholm, 1953, Lundholm, 1956) These workers suggest that the relaxation of smooth muscle by epinephrine is due to an increase in the intracellular lactic acid content, resulting from stimulation of glycolysis in the muscle cell by epinephrine. It can be recalled that a reduction in the tissue pH level is known to have an inhibitory effect on smooth muscle (Evans and Underhill 1924) The hypothesis is based principally on the observations that relaxation of smooth muscle by epinephrine is associated with an increase in the lactic acid content of the tissue, and that metabolic inhibitors of glycolysis block the relaxing effect of epinephrine in such preparations. Furthermore lactic acid alone, in concentrations comparable to those obtained in the presence of epinephrine produces relaxation when added to smooth muscle tissue. If this hypothesis is correct, it is an interesting example of how a drug, by altering the cell metabolism causes the accumulation of a metabolite which inhibits its parent cell by direct action.

B. Direct Action

Measurements of the quantities of drugs necessary to produce an action on cells prove that, in the case of certain powerful agents, the amount fixed to the cell is so small that it can cover only a small fraction of the surface. Furthermore, the speed by which these agents act indicate that they probably exert their effect on the cell membrane. As far as these drugs are concerned, the simplest conception is that they occupy only certain specific active spots or "receptors" on the cell membrane, and that the formation of the drug-receptor complex initiates the observed pharmacological response. Moreover since various agents may produce different types of effect on a single cell it has been suggested that there are specific receptor sites on the cell surface for each kind of drug (Clark, 1937)

The following agents are believed to exert their action by combining reversibly with specific receptors on the surface of smooth muscle acetylcholine, epinephrine, norepinephrine, 5-hydroxytryptamine, pitressin and histamine. Depending upon the type of the smooth muscle tissue, these agents elicit either contracture or relaxation of the muscle preparation. The response of smooth muscle to these drugs is graded, and when the effect is plotted against the dose, a hyperbolic curve is obtained. When the logarithm of the dose is used the curve has

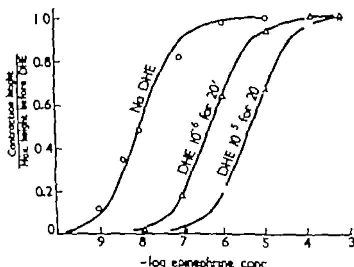


FIG 1 Effect of 20-min. exposure to 10^{-6} and 10^{-5} dihydroergotamine methane sulfonate (DHE) on the response of spiral strips of rabbit aorta to epinephrine (Furchgott, 1955)

a symmetric sigmoid shape. By assuming that the response is directly proportional to the fraction of the total tissue receptors occupied by the drug and that the amount of drug which combines with the receptor is negligible compared to the amount in the surrounding solution, it is possible to fit this dose-response relation into the mass-action formula $K \times X = y/100-y$ where K is a constant different in each case, X is the drug concentration, and y is the biological response in percentage of maximal response (see Clark's extensive review 1937)

A number of agents have been observed to antagonize selectively the effects of the aforementioned drugs. Such antagonists are atropine, which antagonizes acetylcholine dihydroergotamine, which antagonizes epinephrine and norepinephrine and antihistaminics, which antagonize

histamine. These antagonists are believed to combine reversibly with the *same receptors as their agonist, but without evoking a pharmacological response.* If dose-response curves are plotted for the agonist in the presence of these antagonists, the slope of the curves remains unaltered, i e parallel curves are obtained (Fig 1) Antagonism of this type is called competitive on the assumption that antagonist and agonist compete for the same receptor Studies of the kinetics of the development of competitive antagonism and of the recovery from it nevertheless indicate that this

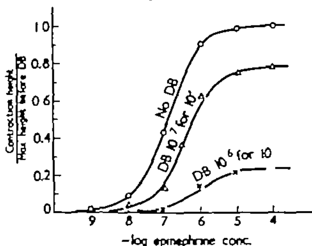


FIG. 2. Effect of 10-min. exposure to different concentrations of dibenamine hydrochloride (DB) on response of spiral strips of rabbit aorta to epinephrine (Furchgott, 1955)

possibly is an oversimplification, and that "drug receptor" interactions are more complicated (Furchgott, 1955) The warning given by Clark (1933) is relevant "Imperfect knowledge appears to be the most probable reason for any apparent simplicity in processes of drug antagonism."

Hypothetically, the antagonist may combine reversibly or irreversibly with a part of the receptor without thereby preventing the agonist from combining with the receptor as well. When however the formation of such an antagonist receptor complex diminishes the pharmacological response produced by the agonist, a situation arises which is known as noncompetitive antagonism. In noncompetitive antagonism the slope of the dose-response curve and the maximal response decline with increasing concentrations of the antagonist. An example illustrating this is *the antagonism produced by β-haloalkylamines* (e g dibenamine) to epinephrine (Fig 2)

(For further information and a quantitative treatment of drug receptor interactions and various forms of antagonism, the following reviews and dissertations should be consulted Clark, 1933 1937 Gaddum, 1937 1943 Schild 1947 1954, Furchgott, 1955 Symposium on Drug Antagonism 1957)

C. Effects of Drugs on the Electrophysiological Properties of Smooth Muscle

Our knowledge of the electrophysiological properties of smooth muscle is still inadequate, and in many instances controversial. This is due chiefly to the great technical difficulties associated with recording of electrical activity from spontaneously contracting tissue, as well as to the smallness of the smooth muscle cell which makes it liable to damage when the intracellular microelectrode technique is used (Bozler, 1946 Bülbring 1954)

It is, however possible to conclude from results obtained by several workers in various smooth muscle tissues that in mammalian preparations, the membrane potential of the cell is 30–80 mv inside negative, and that action potentials are generated by membrane depolarization (Bülbring and Hooton, 1954 Bülbring 1954 Woodbury and Mc Intyre, 1956) A close correlation seems to exist between membrane potential rate of spike discharge, and mechanical tension Results obtained indicate that the membrane potential is inversely correlated to tension whereas the frequency of spike discharge is directly correlated (Bülbring, 1955) Conduction in smooth muscle probably occurs by extrinsic nerve fibers and/or by direct potential spread from cell to cell across sites of low resistance (Bozler 1948 Prosser and Sperelakis, 1956)

Drugs producing increased tension in smooth muscle (acetylcholine and histamine) have been observed to depolarize the cell membrane, and to increase the frequency of spontaneous spike discharge (Fig 3) Epinephrine, on the other hand, when it relaxes smooth muscle, as in the intestinal tract, produces hyperpolarization of the muscle membrane, and decreases the spike discharge (Bülbring 1954 1955)

From the aforementioned observations, it seems likely that drug effects on smooth muscle could be explained by alterations in the resting potential of the muscle cell, i.e. that tension is initiated by membrane depolarization, and that relaxation is initiated by membrane hyperpolarization. The mechanism of drug action is, however prob-

ably more complicated (Evans and Schild, 1957a, b Evans et al., 1958) These investigators have shown that in isotonic KCl or K_2SO solution, various smooth muscle preparations respond qualitatively to drugs, and to some drugs almost quantitatively in the same way as in normal Ringer or Tyrode fluid. An increase in the external potassium concentration of such a magnitude, produces, as could be expected, complete depolarization of the smooth muscle cell Intracellular recording has shown that a membrane potential is not re-established

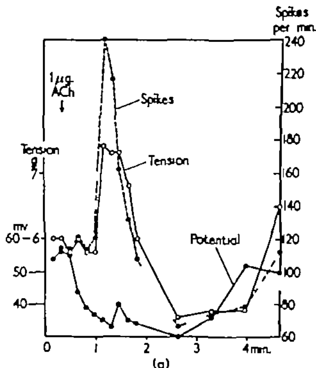


FIG. 3 Effect of acetylcholine on membrane potential •——• rate of spike discharge •– – –• and tension •——• in a taenia coli preparation from the guinea-pig (Bulbring, 1955)

by the drugs. Despite the fact that no potential exists between the inside and the outside of the smooth muscle cell acetylcholine, oxytocin, histamine and 5-hydroxytryptamine elicit a contraction of the muscle preparation of about the same amplitude as in normal Ringer solution and when the muscle is contracted epinephrine causes it to relax Antagonists such as atropine and antihistaminics also retain their activity in potassium Ringer In potassium Ringer solution, the muscle

preparations respond to localized mechanical stimuli by a local contraction, and in normal Ringer fluid by a conducted response. The most feasible explanation offered for these observations is that the contractile response of smooth muscle is primarily initiated by an ionic permeability change in the muscle membrane, and not by a membrane potential change. Another possibility is that drugs acting on the cell membrane causes the release of a specific ion or chemical substance which activates the contractile elements. However in view of the inadequate recording techniques available such theories remain at present only speculations.

D Comments

The introduction of the concept of specific drug receptors at the muscle membrane (Langley 1905, Dale, 1906) and the subsequent quantitative development of the receptor theory (Clark, 1937) have been of fundamental importance for an understanding of pharmacological effects on smooth muscle The receptor theory permits a classification of drug action, and in particular of drug antagonism, and is furthermore of great practical and theoretical importance for the understanding of dose-effect relations. Since it is conceivable that the pattern of the receptors varies slightly in different tissues, the receptor concept also offers an explanation of the finding that the actions of related drugs, as well as drug antagonism, differ with every tissue studied. It should, however be borne in mind that all physicochemical interpretations of cell-drug reactions are still only provisional hypotheses, and not definitely established laws

The bioelectrical approach to the study of drug action in smooth muscle, despite great technical difficulties, has already given promising results. Results hitherto obtained show that the electrophysiological properties of smooth muscle do not differ fundamentally from those of striated muscle, and in particular not from those of the "slow" muscle fiber system. Our knowledge of drug effects on the electrical properties of the cell membrane is still fairly limited, but it is conceivable that a drug-induced muscle contracture is caused by membrane depolarization, whereas relaxation is produced by membrane hyperpolarization The observation that the contractile components of the muscle cell may be activated by drugs, even when no potential difference exists across the cell membrane, is nevertheless of great theoretical interest. It is to be hoped that further elucidation of this phenomenon will

help to clarify the nature of the processes which link events at the cell membrane to changes in the contractile components.

III. EFFECTS OF DRUGS ON STRIATED MUSCLE

For a proper understanding of various drug effects on striated muscle it is necessary to recapitulate briefly some of the main steps involved in neuromuscular transmission and in muscular contraction. A detailed discussion of these processes has been given in Volume II Chapter VI (see also Castillo and Katz, 1956)

i. The arrival of a motor nerve impulse causes the release of acetyl choline from the nerve endings.

ii Acetylcholine enters by diffusion into contact with the post junctional end plate and combines with certain specific structures (the end plate receptors) at the membrane

iii The combination between acetylcholine and the end plate receptors causes the semipermeable end-plate membrane to become highly permeable to *all* ions, and this permeability change leads to a depolarization of the end plate region (the end plate potential)

iv Within a few milliseconds, acetylcholine is removed from the end-plate receptors by enzymatic hydrolysis and by diffusion.

v When the end plate potential has reached a critical amplitude (the "threshold" of the membrane) it activates the adjacent muscle membrane and causes a propagated potential change (the action potential)

vi. The propagated action potential triggers the contractile elements in the fiber and causes muscular contraction by mechanisms the nature of which is unknown

On the basis of this simplified scheme of muscle excitation, it is possible to classify drug action into three groups (a) Drugs interfering with step i i.e. affecting release of the neuromuscular transmitter (b) Drugs acting on the postjunctional end plate membrane involving the events listed in ii–iv (c) Drugs affecting membrane excitability and muscle contraction i e steps v and vi.

A The Release of the Neuromuscular Transmitter

Electron microscopy studies and electrophysiological evidence indicate that the neuromuscular transmitter (acetylcholine) is stored *in the motor nerve endings, probably in intracellular granules or* vesicles, and that a nerve impulse causes disruption of these granules,

with resulting release of a great number of multimolecular units or "quanta" of the transmitter (Robertson, 1956, Castillo and Katz, 1956) The synchronous release of a great number of quanta of acetyl choline produces the end plate potential in the postjunctional mem brane. Even when the myoneural junction is at rest i e no nerve impulse is arriving a spontaneous and intermittent release of discrete "quanta" of acetylcholine occurs. These "quanta" elicit minute potential changes, the so called miniature end plate potentials, which are well below the "threshold" of the fiber (Fatt and Katz, 1952a)

Magnesium and calcium ions have long been known to affect neuromuscular transmission (see review by Engbaek, 1952) That their principal action is on the release of acetylcholine from the motor nerve terminals has, however only recently been established (Castillo and Engbaek, 1954) Using the intracellular microelectrode technique, it could be shown that high concentrations of Mg^{++} ions reduce the number of units of acetylcholine released by each nerve impulse, without a decrease in the amount of the transmitter contained in each unit. The total amount of released acetylcholine is, however, diminish ed and the amplitude of the end plate potential is reduced When the end-plate potential fails to reach the "threshold" of the membrane, a neuromuscular block results. A matter of interest is that Mg ions influence neither the spontaneous frequency nor the amplitude of the miniature end plate potentials i e the effect is strictly limited to the processes responsible for the synchronous transmitter release by a nerve impulse.

Low external concentrations of Ca^{++} ions diminish the output of the neuromuscular transmitter in a way similar to Mg ions High concentrations of Ca^{++} on the contrary increase the number of units of acetylcholine released by each nerve impulse and the end plate potential may reach about three times its normal size (Castillo and Stark, 1952) The spontaneous miniature end plate potentials are not affected by Ca^{++} ions.

An interesting fact is that Ca ions oppose the effect of Mg ions and antagonize their myoneural blocking action. This occurs quanti tatively over a wide range of concentrations, which suggests that they are competitive antagonists at some step in the mechanism responsible for the release of the transmitter by a nerve impulse. It is probable that, between the arrival of a nerve impulse and the release of any individual unit of acetylcholine there is an intermediate reaction

and Katz, 1957a) A possible exception is, however the chronically denervated muscle, in which tubocurarine has been observed to have a slight excitatory (depolarizing) action (McIntyre *et al.*, 1945 Jarcho *et al.*, 1951)

It is considered that tubocurarine blocks neuromuscular transmission by excluding acetylcholine from the end plate receptors and that since the proportional relation between acetylcholine concentration and depolarization still applies in the presence of tubocurarine this



FIG. 4 Effect of tubocurarine on neuromuscular transmission. Records taken at the end-plate region in frog muscle a, before application of curarine: b, c, and d diminution of the initial end-plate potential as tubocurarine action increases. Note progressive lengthening of the latent period of the action potential; e, pure end-plate potential with no action potential, i.e. complete neuromuscular block (Kuffler 1942)

could be due to competitive antagonism (Fatt and Katz, 1952b) On the other hand, it has been shown that the rate of dissociation of the curare-receptor complex is slow and probably does not allow acetylcholine—on the basis of simple competition—to replace curare at the receptors within the short period observed experimentally (Castillo and Katz, 1957a)

When tubocurarine is applied in progressively increasing concentrations to the muscle, its first effect on the previously curare-free preparation is a shortening of the duration of the end plate potential. This is followed by a gradual depression of its amplitude and its rate of rise is reduced (Eccles *et al.*, 1941) Finally the end plate potential is completely abolished. When the amplitude of the end plate potential is below the "threshold" of the membrane, a neuromuscular block occurs (Fig 4) Elimination of the drug causes rapid and full recovery of transmission.

Because of the competitive mode of behavior any local increase in transmitter concentration will antagonize the curare block. Repetitive motor nerve stimulation, known initially to increase the acetylcholine release, thus has a short lasting anti-curare action. Drugs which inactivate cholinesterase are effective curare antagonists, since they prevent the enzymatic destruction of acetylcholine, and thus increase its concentration at the end plate region (see also Riker 1953)

Since a neuromuscular block results when the amplitude of the end plate potential is below the "threshold" of the membrane, any reduction in the "threshold" will antagonize the block. Increased membrane excitability is produced, for instance, by a "subthreshold" depolarization of the muscle fiber and provides an explanation of the anti-curare effect of cathodal currents and of an elevated external K ion concentration. Similarly agents which raise the membrane threshold" will intensify a curariform block.

In addition to the curare alkaloids, numerous synthetic agents have a non-depolarizing blocking action on neuromuscular transmission. Although these compounds presumably react with the same receptors as tubocurarine, they do not necessarily behave as competitive antagonists to acetylcholine. Consequently drugs producing cholinesterase inhibition do not invariably antagonize their neuromuscular blocking action

b Depolarizing Drugs Like acetylcholine, a number of choline esters and quaternary ammonium compounds produce a depolarization of the end-plate region Among the compounds with this action are

resistance and of the time constant of the membrane in the repolarized end plate region (Thesleff 1955a 1956a) Both were found to be normal consequently it seems unlikely that repolarization of the end plate membrane is to be ascribed to permeability changes of the aforementioned type.

The most probable explanation, therefore is that depolarizing drugs reversibly change the end plate receptors from the normal type to an inert acetylcholine insensitive type, which fails to maintain the high ionic permeability in the end-plate membrane. (For a discussion of possible drug receptor interactions see Katz and Thesleff 1957a)

The change in the drug receptor complex from an active to an inactive form occurs with physiological concentrations of acetylcholine and has a time-course related to drug concentration. High concentrations of acetylcholine produce "desensitization" of the end-plate receptors with a half-time of development of a few seconds or less (Katz and Thesleff 1957a Axelsson and Thesleff, 1958)

The recovery time from "desensitization" varies considerably with experimental conditions. When a depolarizing agent is applied locally at close range to a single end plate region of a frog muscle fiber and is allowed to produce "desensitization" of the receptors, the recovery time after withdrawal of the drug is roughly independent of the applied drug concentration and has a half time of about 5 sec. If however the drug is applied to the whole muscle and left to diffuse into it, the recovery in a single fiber is much slower (> 30 min.) This discrepancy in recovery time between various forms of drug application is poorly understood, and is probably an indication that hitherto undisclosed slow changes may occur in the "desensitized" receptor

A neuromuscular block produced by depolarizing agents is in many instances not antagonized by cholinesterase inhibitors. The presence of a non-depolarizing blocking agent such as curare antagonizes the depolarization produced by depolarizing agents but enhances the subsequent "desensitization" block (Zaimis, 1953)

Some myoneural blocking agents, for instance certain ethyl analogs of decamethonium, produce a depolarization far less conspicuous than that caused by the aformentioned drugs (Thesleff 1955b) Whether these compounds should be classified as non-depolarizing or as depolarizing blocking agents is a matter of opinion and has little significance as long as it is not known whether a fundamental difference exists between the two forms of myoneural block (see however Grundfest, 1957)

2 *Anticholinesterase Drugs*

One of the main factors responsible for the rapid removal of acetyl choline from the end plate receptor is the presence of an enzyme system, cholinesterase, capable of destroying acetylcholine The distribution of this enzyme in the muscle and its rôle in neuromuscular transmission are discussed in Volume II Chapter V

Numerous agents from all classes of pharmacologically active drugs have been reported to inhibit cholinesterase when present in a sufficiently high concentration It is however customary to designate as anticholinesterase drugs only those compounds which are potent cholinesterase inhibitors, and which exert their pharmacological effects mainly by an inactivation of these enzymes.

The pharmacological response of striated muscle to drugs inhibiting the enzyme cholinesterase are due mainly to delayed destruction of the transmitter substance. At the resting myoneural junction, this is manifested as an increase in amplitude of the spontaneous miniature end plate potentials (Fatt and Katz, 1952a) In the presence of a cholinesterase inhibitor these potentials may reach an amplitude of several millivolts, and if many of them are summated they will produce a propagated action potential. This causes spontaneous twitches in individual muscle fibers (a phenomenon known as fibrillation) and is commonly observed in mammalian muscle in which the cholinesterase has been inactivated (Liley 1956) There is some indication that anticholinesterase drugs also increase the spontaneous frequency of the miniature end plate potentials and thus facilitate synchronous discharge of several units of acetylcholine (Fatt and Katz, 1952a Nastuk and Alexander 1954) Furthermore, it is possible that intramuscular nerve branches are excited by the currents generated in the activated muscle fibers, and that motor nerve impulses thereby also participate in producing fibrillation (Eccles *et al.*, 1942 Lloyd 1942)

When acetylcholine is released by a nerve impulse its action is enhanced by cholinesterase inactivation. The result is an increased amplitude and a striking prolongation of the end plate potential (Fig 6) The lengthy depolarization of the end-plate acts as a local "sink," to which current flows from the surrounding membrane. This may produce repetitive firing of the membrane i.e. a tetanic twitch in response to a single nerve impulse. Repetitive motor nerve stimulation at high frequency on the other hand, produces a neuromuscular block

(known as Wedensky inhibition) which is presumably due to local accumulation of acetylcholine at the end plate. It therefore has the characteristics of a block produced by a depolarizing agent.

With most cholinesterase inhibitors the rate of formation of the inhibitor-enzyme complex is fairly slow and has a time constant of several minutes (Augustinsson and Nachmanson 1949 Goldstein, 1951) An exception however, is edrophonium (2 hydroxy phenyl dimethylethylammonium chloride) which rapidly inactivates the

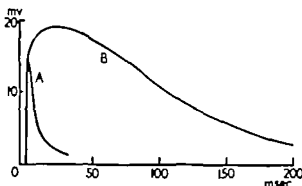


FIG. 6. Effect of neostigmine on the end-plate potential in a single muscle fiber of the frog. Superimposed tracings of end-plate potentials, A, in an uncurarized muscle with transmission blocked by a reduced sodium concentration and B, after addition of 10^{-6} w./v. neostigmine (Fatt and Katz, 1951)

cholinesterase and has an almost instantaneous anti-curare action (Nastuk and Alexander 1954) In frog muscle the time constant for formation and dissociation of the edrophonium-enzyme complex is less than 0 1 sec. (Katz and Thesleff 1957b)

In sufficiently high concentrations, most—if not all—cholinesterase inhibitors also have a direct effect on the acetylcholine receptors of the end-plate. Depending on their chemical structure, this is manifested either as a non-depolarizing or as a depolarizing blocking action. This probably accounts for the differences between the anti-curare action of various equipotent cholinesterase inhibitors (Riker 1953) A number of neuromuscular blocking agents, for example decamethonium, have on the other hand anticholinesterase activity which may influence their interaction with the neuromuscular transmitter at the end plate region (Castillo and Katz, 1957b)

C. The Excitability of the Muscle Fiber

A multitude of drugs exert a direct action on the excitability of the muscle membrane and thereby alter the size and shape of the muscle twitch (see reviews by Gasser 1930 Hunt and Kuffler 1950) It is possible in this chapter to include only a few of these agents. For the sake of uniformity in presentation only those drugs will be discussed that have been shown to exert their action mainly on the excitatory system of the muscle membrane.

Depending on the recording technique, these drugs have been observed to change either the electrical properties of the fiber mem brane, or when tension recording is used the "active state" of the muscle. Both effects originate in the muscle membrane but since there is no conclusive evidence of a relation between the electrical and the mechanical changes occurring in muscle, they will be treated sepa rately

1 *The Electrical Properties of the Muscle Membrane*

A single stimulus to a muscle which, under normal circumstances, causes a single response will, in the presence of veratrum alkaloids, give rise to a prolonged after-discharge or series of repeated action potentials (Kuffler 1945) This repetitive response to a single stimulus is observed only when veratrine is applied locally to a muscle fiber, and disappears when the whole fiber is soaked in veratrine (Burns *et al.*, 1955) The explanation of this effect is that veratrine delays the repolarization phase of the action potential, thereby producing the flow of an electric current from the neighboring veratrine-free and already polarized membrane. This current depolarizes the normal membrane and sets up a second action potential, which causes both veratrinized and normal membrane to depolarize and the cycle of events can again be repeated (Burns *et al* 1955) In frog muscle, the time constant of the veratrine induced delay of repolarization may be as great as 5 sec. The mechanism by which veratrine delays repolarization in unknown. (For a review of the general pharmacology of veratrum alkaloids and their action on various muscle tissues, see Krayer and Acheson, 1946)

The tetraethylammonium ion has an action similar to the aforementioned (Hagiwara and Watanabe 1955) Like veratrine this quaternary ammonium ion prolongs the duration of the action poten

tial and gives rise to a repetitive discharge following a single stimulus. Determinations of the membrane resistance during the prolonged decay of the action potential have shown that the change was less marked than that observed during the decay of a normal action potential. Consequently a possible interpretation is that the prolongation of the action potential is due to a diminished and delayed change in potassium conductance in the active membrane.

In contrast to the above drugs, a number of agents diminish the excitability of the muscle membrane Hypnotic drugs like the barbituric acid derivatives, paraldehyde urethane, chloral hydrate, chloralose, and tribromethanol have been shown to increase the electrical "threshold" of the muscle membrane, and to reduce and finally to abolish the action potential (Quilliam, 1955a, b Thesleff 1956b) The rate of rise of the action potential is reduced and its duration is prolonged These changes occur without a significant alteration in resting membrane potential or in ionic permeability of the membrane The reduced membrane excitability can therefore probably be ascribed to an inhibitory action of hypnotic agents on the mechanism responsible for the selective sodium permeability increase initiating the action potential (Thesleff 1956b) Local anesthetic agents presumably have a similar mode of action (Weidmann, 1955 Straub 1956)

2. *The "Active State" of Muscle*

Following an adequate stimulus, tension develops in a striated muscle and it contracts. The process underlying contraction is known as the "active state" (Hill 1949) Because of the elastic elements in the muscle fiber the duration of the muscle twitch is much longer than that of the "active state " and the latter is normally too brief to allow development of a maximal twitch tension. Prolongation of the "active state" by for instance repetitive stimulation of the muscle or by drug action gives the elastic elements time to develop a higher tension, and thereby to increase the mechanical response of the muscle (for a short review see Wilkie 1956)

Development of the "active state" following excitation occurs rapidly and is probably triggered by a physicochemical process originating in the membrane and then propagated inwards. The nature of the process which links the membrane with the contractile components in the muscle fiber is not known. It is possibly associated

with the changes in the electrical properties of the membrane accompanying the action potential but such a relation has not been established (Huxley 1957)

A number of drugs have been shown to change the time course of the "active state" without otherwise altering the contractile mechanism. Such agents are adrenaline and caffeine (Goffart and Ritchie, 1952), nitrate, bromide, and iodide ions (Hill and Macpherson, 1954 Kahn and Sandow 1955) quinine and quinidine (Lammers and Ritchie, 1955) tetraethylammonium and tetramethylammonium ions and choline (Edwards *et al* 1956) By determinations of the "active state" curve in muscle, all these agents could be shown to delay the falling phase of the curve, and thus to prolong the duration of the "active state." The twitch response to a single stimulus was correspondingly increased whereas the tetanic tension remained unchanged

A matter of interest is that all these agents have also been found to prolong the duration of the action potential its falling phase lasts long enough to be reasonably well correlated in duration with the "active state" curve (Edwards *et al.*, 1956) As previously mentioned, hypnotic agents increase the duration of the action potential and are also known to augment the twitch response to a single stimulus (Kraatz *et al.* 1953) Their action on the "active state" curve has not, however yet been studied

D The "Slow" Muscle Fiber System of the Frog

The "slow" muscle fiber system consists of small motor nerve fibers conducting at 2–8 m. per second, and of their "slow" muscle fibers which are quite distinct from the previously considered twitch fibers. No propagated impulse can occur in a "slow" fiber the motor nerve sets up *local* potentials at the junctions which in turn cause local contractions. Since the "slow" fibers possess numerous junctions along their whole course, practically simultaneous activation of the muscle fiber takes place (Kuffler and Vaughan Williams, 1953a, b)

"Slow" muscle fibers are present in the striated musculature of the frog and occur in particular abundance in the iliofibularis and in the rectus abdominis muscle A "slow" fiber system comparable to that in amphibians has not been demonstrated in mammalians.

When a muscle with "slow" fibers (e.g musculus rectus abdominis) is immersed in Ringer fluid containing drugs like acetylcholine or KCl, tension develops and muscle shortening follows The contracture is

tial and gives rise to a repetitive discharge following a single stimulus. Determinations of the membrane resistance during the prolonged decay of the action potential have shown that the change was less marked than that observed during the decay of a normal action potential Consequently a possible interpretation is that the prolongation of the action potential is due to a diminished and delayed change in potassium conductance in the active membrane.

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When a muscle with "slow" fibers (e.g musculus rectus abdominis) is immersed in Ringer fluid containing drugs like acetylcholine or KCl tension develops and muscle shortening follows. The contracture is

graded depending on drug concentration and is roughly maintained during the period of drug application. A similar effect is produced by a cathodal current through the muscle while relaxation of the muscle occurs at the anode. Drug induced muscle contractures are intensified or relieved by a cathodal and an anodal current, respectively It has been shown that contractures are initiated by membrane depolarization and that graded depolarization gives a graded tension response. In contrast to the twitch fiber system, which relaxes relatively rapidly despite continued membrane depolarization, the "slow" fibers stay contracted during the whole period of reduced membrane potential (Kuffler 1946 Fleckenstein *et al* 1951 Kuffler and Vaughan Williams 1953a, b Fleckenstein, 1955)

The depolarization produced by acetylcholine approaches the zero potential level, as can be excepted in a membrane which is simultaneously highly permeable to K (or Cl) and Na ions (Burke and Ginsborg 1956) The effect of acetylcholine on the "slow" fiber membrane is thus similar to that observed at the end plate region of twitch fibers. It is not known whether the depolarization produced by acetylcholine is maintained in the presence of the drug but the observation that the muscle remains contracted, and that anodal currents produce relaxation, suggest that the membrane is persistingly depolarized

Acetylcholine, in contrast to K ions, is believed to act on specific membrane receptors located at the numerous junctional regions of the "slow" muscle fiber This assumption is strengthened by the observation that junctional blocking agents like tubocurarine and atropine antagonize the effect of acetylcholine without altering the depolarizing action of K ions.

The antagonism produced by atropine and curare alkaloids to acetylcholine is quantitative over a wide range of concentrations, which suggests that they are competitive antagonists (Clark, 1937 van Maanen, 1950)

In view of the graded contracture of "slow" muscle fibers to increasing concentrations of depolarizing drugs as well as the selective antagonism produced by certain agents, such muscle preparations are eminently suitable for acetylcholine assays and for studies of competitive and noncompetitive antagonism (Clark, 1937 Ariens *et al.* 1956)

E. The Intrafusal Fibers of the Muscle Spindle

In mammals small motor nerve fibers innervate the intrafusal muscle fibers of the proprioceptive muscle spindle (Leksell, 1945) Histologically this innervation is multiple i.e., one intrafusal fiber possesses several

motor end plates (Barker 1948) Moreover physiological evidence suggests that the intrafusal fibers of the mammalian spindles are capable of slow graded contractions (Kuffler *et al.* 1951) These properties point to an analogy between the "slow" muscle fiber system of the frog and the intrafusal fibers of the mammalian muscle spindle (Kuffler and Vaughan Williams, 1953b Granit, 1955) Recent electrophysiological investigations have, on the other hand, shown that propagated action potentials occur in the intrafusal fibers of the frog muscle spindle consequently it is probable that at least some of these fibers have "twitch" fiber properties (Eyzaguirre, 1957 Buchthal and Jahn, 1957)

Despite the great importance of the proprioceptive muscle spindles for maintenance of co-ordinated muscle movements, few studies have been made of their response to different drugs. Acetylcholine increases the sensory discharge from mammalian muscle spindles presumably by producing contracture of their intrafusal muscle fibers This acetyl choline effect is blocked by tubocurarine and enhanced by physostigmine (Hunt, 1952) Agents like succinylcholine and decamethonium probably also depolarize the intrafusal muscle fiber membrane The former agent has been shown to elicit a frequent and regular sensory discharge from the muscle spindle, even in concentrations which do not affect the twitch fiber (Granit *et al.* 1953) Besides its depolarizing action on the intrafusal muscle fibers, it probably also has a direct excitatory effect on the sensory nerve endings [cf the stimulatory effect of acetyl choline on sensory receptors (Landgren *et al.* 1954 Diamond, 1955)] The Golgi tendon organs are not affected by depolarizing agents. In low concentrations, tubocurarine produces brief stimulation of the muscle receptor whereas in higher concentrations it blocks the response to depolarizing drugs The concentration of tubocurarine required to block the intrafusal muscle fibers is higher than that which produces a neuromuscular block in ordinary twitch fibers (Granit *et al.*, 1953)

Any change in the normal function of the intrafusal muscle fibers tends to alter or abolish the autogenetic stretch reflex, and thereby to interfere with the servo control exerted by the muscle spindles on voluntary muscle movements. This aspect of drug action has not, however been elucidated.

F Comments

Recent biophysical methods in particular the recording of electrical phenomena from single cells, offer excellent opportunities for detailed studies of the effects of drugs on striated muscle. Such investigations are

particularly valuable when the drug effect can be analyzed into elementary physical or physicochemical reactions. This has been possible in many instances, and our understanding of the mode of drug action has thereby been considerably promoted. Large gaps nevertheless still exist in our knowledge of the pharmacology of striated muscle. Interest in the application of biophysical techniques is, however rapidly growing and there is every reason to assume that our knowledge in this field of pharmacology will increase.

The release of the neuromuscular transmitter by a nerve impulse is reduced by Mg^{++} low Ca^{++} ion concentrations, and by botulinum toxin. High external concentrations of Ca^{++} ions counteract, presumably by competitive antagonism the effects of Mg ions. Neither of these ions influence the transmitter storage capacity or the spontaneous quantal transmitter release from the prejunctional structures. Their effect is strictly limited to the processes responsible for the transmitter release by a nerve impulse. Botulinum toxin, on the contrary has the unique ability to block the spontaneous quantal ejection mechanism as well. The mode of causation of these changes is and will remain obscure until the mechanism by which nerve impulses liberate or facilitate the release of acetylcholine is revealed.

According to whether or not they depolarize the end plate region postjunctional neuromuscular blocking agents have been classified as either depolarizing or as non-depolarizing agents. The belief that depolarizing agents render the muscle fiber locally inexcitable by excessive, maintained depolarization seems, however to be erroneous. The depolarization is generally only a transient event, which very rapidly passes into a non-depolarizing inhibition, i.e. into a neuromuscular block in which the electrical properties of the muscle membrane are normal In the opinion of the author the postjunctional neuromuscular block produced by depolarizing agents is due to the formation of an inert drug receptor complex, which greatly resembles that formed by non-depolarizing (curare-like) agents. According to this view it is scarcely possible at present to make a clear distinction between the neuromuscular block produced by a non-depolarizing agent and the block produced by a depolarizing one.

Numerous agents affect the direct excitability of the muscle membrane In most instances however their mode of action is unknown An interesting feature is that drugs like hypnotic and anesthetic agents diminish the muscle membrane excitability just as they depress

synaptic transmission in the central nervous system. Consequently a systematic study of the effects produced in the muscle membrane could probably serve as a guide to the mechanism or mechanisms by which these agents depress the neuronal cell membrane. Presumably drug-induced changes in the electrical properties of the muscle membrane would, if combined with analytical studies of the mechanical response of the muscle, also provide valuable information regarding the processes by which excitation is transmitted from the cell membrane to the contractile components in the interior of the fiber

The small nerve-"slow" muscle fiber system of the frog has been the subject of a large number of pharmacological investigations. Unfortunately most of these studies were made long before the distinct physiological properties of this nerve-muscle system were realized. It is now difficult to interpret the results, and many of these investigations will have to be repeated. Drug induced contractures in "slow" muscle fibers, however seem to be related to corresponding depolarizations of the muscle membrane. Whether or not depolarization is invariably maintained throughout the contracture is not known, nor have the mechanisms been elucidated by which agents other than acetylcholine depolarize the membrane.

Evidence indicates that the intrafusal fibers of mammalian muscle spindles are functionally related to the "slow" fiber system of the frog Very little, however is known about their actual response to various drugs. In view of the observation that the sensory discharge from the muscle spindle is proportional to the degree of stretch of the intrafusal fibers, there seems to be a serious flaw in our pharmacological knowledge. A drug selectively blocking the intrafusal muscle fibers could probably be of therapeutic value in those states of muscle rigidity and spasticity which are believed to be due to a pathological continuous afferent discharge from the muscle spindles.

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CHAPTER II

The Effects of Drugs on Myocardial Contractility

NEIL C. MORAN AND MARION DEV. COTTEN

I. INTRODUCTION

When the heart is considered as a muscular structure, the function of which is the coordinated pumping of blood through the vascular system, it is not unexpected to find that drugs can produce a variety of complex changes in its activity. Through direct and indirect mechanisms, drugs are capable of altering not only the contractile activity of the myocardium but also the rate of pacemaker discharge, the speed of impulse conduction and the rhythm of the heart. Adding to this complexity is the large number of diverse chemical substances which alter cardiac funtion.

Refined analysis of physiological and pharmacological aspects of cardiac activity has become possible only with the introduction of

adequate experimental techniques, beginning in the latter part of the last century This has resulted in the accumulation of an extensive literature devoted to studies of the cardiac actions of many natural and synthetic chemical compounds. Most of this literature consists of merely descriptive pharmacology although recently there has been increased emphasis on determining the fundamental mechanisms of drug action at cellular and subcellular levels.

Because a comprehensive analysis of the subject of cardiac pharmacology would be too extensive, this chapter will be limited to a discussion of the effect of selected drugs on myocardial contraction. The effects of pharmacological agents on electrophysiological properties of the heart, such as excitability conduction, and automaticity, will be omitted, although their importance is recognized Readers are referred to a recent monograph by Brooks *et al.* (1955) for discussions of this latter topic. Furthermore, the omission of discussion of certain cardiac stimulants, such as the xanthine derivatives (caffeine, theophylline, and theobromine) and cardiac depressants such as quinidine and barbiturates, has been deliberate because of the nearly total absence of work on basic mechanisms of action of these drugs on cardiac contractility

The locus of action and the basic mechanisms by which drugs alter the contraction of heart muscle are of primary importance in understanding their actions. Theoretically drugs can influence myocardial contraction in several ways. They may alter the supply of energy for contraction either at the stage of final utilization or at some stage in the sequence of production and transfer of energy They may directly affect the contractile proteins. Finally they may influence the coupling of the wave of excitation at the cell membrane with mechanical contraction. These potential mechanisms obviously are not mutually exclusive and the possibility exists that drugs may influence the contraction of heart muscle by a simultaneous effect on two or more mechanisms. Precise localization of the site of action of a drug is dependent on an understanding of the intimate details of muscle contraction, knowledge of which is still incomplete.

Many drugs affect myocardial contractility both directly and indirectly Complete analysis should account for both types of action. Indirect effects include (a) release from extracardiac sources of humoral substances which when transported to the heart, influence the force of contraction, e.g epinephrine from the adrenal medulla (b)

alterations in the autonomic regulation of the heart, (c) hemodynamic changes, which by altering myocardial fiber length, change contractile force, and (d) alteration of the blood supply to the myocardium.

The evaluation of the effects of drugs on myocardial contractility is dependent on an appreciation of the limitations of the methods employed. These methods vary from measurement of the contraction of extracted actomyosin threads or of isolated segments of myocardium to indirect determinations of cardiac output and cardiac work in intact animals or human subjects. For a detailed discussion of many of the methods used in animal experimentation, see Loubatieres (1951)

Ideally, it would be desirable to directly measure changes in force of contraction of the myocardial fibers. Such evaluation is possible with isolated hearts of animals, with isolated segments of hearts and as the result of recently developed techniques, with hearts *in situ* in animals. These latter methods employ the use of resistance strain gauge equipment and have been described by Walton *et al* (1950a b) and Boniface *et al* (1953) The validity of the measurement of cardiac contractile force *in situ* by the use of resistance strain gauges has been demonstrated by Cotten (1953), Cotten and Bay (1956) and Cotten and Maling (1957)

It is not always feasible, however to measure contractile force directly and reliance then must be placed on measuring changes in cardiac functions which result from changes in ventricular contractile force. With these methods, care must be taken to control or to account for changes in secondary factors such as heart rate, cardiac size, arterial pressure, and venous return of blood to the heart, as these are factors which may indirectly alter cardiac contractile activity Among the indirect methods are measurements of changes of intraventricular pressures changes of cardiac output, changes of the size of the heart, and calculated changes of ventricular stroke work. Wiggers (1927a) has presented an excellent evaluation of the analysis of cardioactive drugs based upon changes of intraventricular pressures. For examples of the use of changes of cardiac output and ventricular stroke work, see Sarnoff *et al* (1954) and Cotten and Maling (1957) Rushmer (1955) and Rushmer and West (1957) have utilized several ingenious methods for measuring changes in the size of dog hearts and for the evaluation of the function of the heart based upon analysis of cardiac work and power The use of the electrokymograph, a radiologic technique for measuring changes in the size of the heart in man (Heyer and Boone

1952) has been employed by Eddleman *et al* (1951) and by Haring and Luisada (1953) for studying the effects of drugs in man.

II. CARDIAC GLYCOSIDES

Among the most important drugs employed in medical practice is a large group of closely related compounds known as cardiac glycosides. In the form of Galenical preparations, they have been used for centuries, but modern understanding of their actions began in 1785 with the classic description by William Withering of the beneficial effects of the foxglove (*Digitalis purpurea*) in patients with dropsy Although Withering attributed most of the action of digitalis to an effect on the kidney it is recognized today that the edematous patients he treated were suffering from congestive heart failure and that the diuretic effect of digitalis was largely secondary to the increased cardiac output which the drug produces in this state. Nevertheless, Withering also recognized that digitalis has important actions on the heart, actions largely overlooked for the next hundred years.

Because the literature relating to cardiac glycosides is too large to review completely in this chapter only those papers will be cited which, in the opinion of the authors, are most helpful in understanding the effects of these compounds on myocardial contractility The older literature has been adequately reviewed in several monographs on cardiac glycosides (Cushny 1925 Kisch, 1944 Movitt, 1949) A review in the French literature covers much of the work of their effects on contractility (Loubatierres, 1951) and a recent review by Hajdu and Leonard (1959) critically examines the cellular basis of action of cardiac glycosides.

The term cardiac glycoside will be used in a general sense to apply not only to the glycosides but also to certain non-glycosidic compounds which either are derived from the glycosides or occur in nature without the sugar components.

A. Sources and Chemistry

Cardiac glycosides are present in a variety of plants but are obtained mainly from *Digitalis purpurea* *Digitalis lanata* *Strophanthus* species, *Urginea* species (Squill) and *Convallaria* species. In addition secretions of the skin and salivary glands of toads contain non-glycosidic compounds resembling plant glycosides in pharmacological action and in fundamental ring structure.

The glycosides are composed of one or more molecules of monosaccharide sugars attached to a cyclopentanoperhydrophenanthrene nucleus. At position 17 of this steroid nucleus (known also as a genin or *aglycone*) is attached an unsaturated lactone ring The lactone ring may be five-membered as in the digitalis and strophanthus glycosides or six membered, as in glycosides from squill and in cardioactive steroids from toad venom. In addition to the sugars and lactone ring other groups, such as hydroxyl, methyl aldehyde, and acetyl, are attached to the steroid ring at various positions. The sugar molecules are not essential for pharmacological activity although they augment potency presumably by enhancing water solubility and cell penetrability of the aglycone. Although semi-synthetic compounds have been made, complete synthesis of the aglycones or glycosides has not been achieved. The chemistry of the cardiac glycosides has been comprehensively reviewed by Stoll (1949)

B. Effects of Glycosides on Myocardial Contractility

The cardiac glycosides and related aglycones directly augment the contractility of the myocardium. In addition, they influence cardiac activity through indirect actions such as vagal slowing of rate, depression of speed of propagation of impulses through the cardiac conduction system, changes in ventricular excitability initiation of cardiac arrhythmias, and alterations in peripheral resistance and venous return to the heart.

1 *Direct Action on Contractile Force*

All of the active cardiac glycosides and aglycones increase the magnitude and rate of development of systolic contraction and are capable of increasing ventricular work. These effects have been demonstrated repeatedly by many investigators using a variety of experimental techniques.

Indirect evidence for increased contractile force has been obtained from at least two different types of experiments. Plant (1914) utilizing the heart lung preparation for the study of cardio-active drugs, found that strophanthin increases cardiac output, reduces atrial pressures, and slightly increases arterial pressure without changing heart rate. In this preparation, with controlled systemic arterial resistance and venous return these effects are a result of increased ventricular contractile force. Many investigators have confirmed Plant s observations

on the heart lung preparation (Krayer 1931 Peters and Visscher 1936 Farah and Maresh 1948 and others) The use of ventricular function curves obtained from calculated stroke work and atrial pressures in dogs with intact circulatory systems also shows that the work capacity of the ventricles is enhanced by ouabain (Cotten and Stopp 1958)

A second indirect method demonstrating augmented myocardial contractility is the measurement of changes in intraventricular pres-

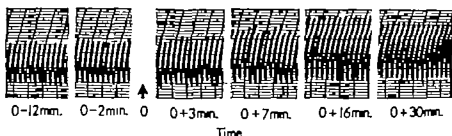


FIG. 1 The effect of a cardiac glycoside on right ventricular force in an unanesthetized dog. A strain gauge arch was sutured to the ventricle several days before this experiment. Atropine sulfate, 1 0 mg./kg., was given parenterally before injection of the glycoside, to eliminate vagal slowing of the heart. Morphine sulfate, 1 0 mg./kg., was also given to sedate the animal. Lanatoside C, 0.05 mg./kg., was injected intravenously over a two minute period beginning at zero time (at arrow) The amplitude of excursion of the oscillograph pen bears a linear relationship to force of contraction. Maximum effect (an increase of about 50%) was obtained 16 min. after beginning of injection. From Walton *et al.* (1950a) through the courtesy of the authors and *The Journal of Pharmacology and Experimental Therapeutics.*

sures in animals. In 1927 Wiggers and Stimson found that, in dogs, strophanthin and a crude digitalis mixture increase maximal intraventricular systolic pressures and the rate at which the pressures rise during isometric contraction. They also noted shortening of the period of systolic contraction. These results, obtained under conditions of controlled heart rate and arterial pressure, indicate an increase in ventricular contractile force.

A decade later Cattell and Gold (1938) established the fact that glycosides directly increase isometric contractile force of the hypodynamic papillary muscle of the cat. This phenomenon has been confirmed by other workers using cat papillary muscle (Weeks and Holck 1943 White and Salter 1946 White *et al* 1948) rat ventricle strips (Maruoka and Saunders, 1950) tortoise ventricles (Wedd *et al* 1941), guinea pig auricles (Furchgott and Sleator 1954) and papillary

muscle from the dog and human being (Loubatieres, 1951) White et al (1948) systematically surveyed several different glycosides and found that they are all qualitatively alike in their action on cat papillary muscle

More recently Walton et al (1950a) using strain gauge recording systems, have shown that several cardiac glycosides increase ventricular contractile force of the dog heart in situ (Fig 1) Loubatieres (1951), using a spring lever system in dogs, and the authors (Cotten and Moran, unpublished observations) using the strain gauge arch in dogs, cats, and monkeys, have obtained comparable results.

In confirmation of the observations of Wiggers and Stimson (1927) that glycosides shorten the duration of systole, Garb and Chenoweth (1949) demonstrated that digitoxin shortens systole in the cat papillary muscle. Also Cotten and Bay (1956) found that lanatoside C accelerates the development of ventricular contractile force in dogs an effect correlated with the abbreviated isometric pressure gradient produced by glycosides.

2 *Influence on Diastolic Tone*

The frequently observed decrease in size of the dilated ventricles following administration of cardiac glycosides to patients with congestive heart failure has led to the concept that glycosides increase diastolic tone of the heart. This concept is supported by the experimental work of Luisada and Diamond (1955) who found that many glycosides increase resting tension of segments of dog hearts, and of Loubatieres (1951) who described increased diastolic tension in cat and human papillary muscle (but a decrease in dog papillary muscle) However other workers have found no evidence for such an effect. In the experiments of Wiggers and Stimson (1927) end-diastolic intraventricular pressures were unchanged by doses of strophanthin which increased maximal intraventricular systolic pressures. Kabat and Visscher (1939) found no effect on diastolic elasticity of actively contracting tortoise ventricles after providing doses of glycosides which increased cardiac systolic tension and work. The diastolic tension of frog ventricle strips (Lundin and Strom, 1948) and of rat ventricle strips (Benforado 1958) is unchanged by doses of ouabain which increase contractile force. While this question has not been answered conclusively it would appear that a change in diastolic tone of the heart is not an important action of the glycosides. The decrease in size

of the failing heart in patients treated with these drugs can be explained adequately by (a) the increased emptying of the heart due to the augmented contractile force, and (b) a reduction of circulating blood volume.

3 *Effects on Failing and Nonfailing Hearts*

Cardiac glycosides, according to a widely held concept, increase contractile force only of failing myocardium. This concept stems from the belief that congestive heart failure is due to a defect of some phase of the contractile process which is corrected by glycosides (Olson and Schwartz, 1951 Bing, 1955) and from frequent observations that glycosides do not increase cardiac output in normal man or animals. Certain experimental results of studies of tortoise hearts (Wedd *et al.*, 1941 Wedd and Blair 1948) tend to support this point of view

However, recent data derived from studies on man, dogs, and isolated ventricle strips of the rat cast doubt on the validity of this concept. Eddleman *et al.* (1951) on the basis of electrokymographically observed decreases in systolic and diastolic size of the heart and acceleration of cardiac ejection in normal man following intravenous injections of glycosides, concluded that the drugs augment ventricular contractility but that peripheral actions resulting in reduced venous return of blood to the heart prevent translation of the augmented force into effective work. Similar results were obtained by Haring and Luisada (1953)

In both anesthetized open-chest dogs and conscious dogs (in which a strain gauge arch has been attached to a ventricle at a previous operation) intravenous injections of a number of glycosides produce substantial increases in contractile force (Walton *et al.* 1950a) See Fig 1 Neither the anesthetized nor the conscious dogs showed signs of cardiac failure.

Sanyal and Saunders (1957) have demonstrated that glycosides increase the contractile force of "nonfailing" isolated ventricle strips of the guinea pig When placed in a bicarbonate buffered medium and driven by rhythmic electrical stimulation, these strips show no sign of fatigue over a period of several hours. Addition of ouabain increases contractile force. When phosphate buffer is used (as was employed by Cattell and Gold, 1938) the contractile force of the strip progressively declines with the passage of time. Ouabain, administered immediately after the preparation is set up or at any time during the progressive

failure causes substantial increases in contractile force. Figure 2 shows the results of these experiments. These observations have been confirmed in cat papillary muscle (Cotten, unpublished observations) and in rat ventricle strips and isolated atria of rabbits (Moran, unpublished observations)

The observations that glycosides do not increase cardiac output or ventricular stroke work in normal animals (Harrison and Leonard, 1926 Olson *et al* 1955 Regan *et al*, 1956) must be reconciled with

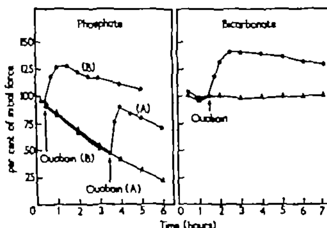


FIG. 2. The effect of ouabain on contractile force of isolated, electrically-driven strips prepared from right ventricles of guinea-pigs. In the left-hand illustration, strips were placed in a phosphate-buffered medium. The graph Δ—Δ shows the progressive decrease in contractile force which occurs with time in this medium. The graph •——• (A) shows the effect of ouabain in a concentration of 1:6,000 000 on the contractile force of a hypodynamic strip, while graph •——• (B) shows the effect of the same concentration of ouabain in another strip before development of the hypodynamic state. In the right-hand illustration, strips are placed in a bicarbonate-buffered medium which prevented development of the hypodynamic state (graph Δ—Δ) The administration of ouabain (1:6,000,000 or 2.3×10^{-7} M) at any time during the interval of the experiment increased contractile force as illustrated by graph •——• From Sanyal and Saunders (1957) through the courtesy of the authors and the *Proceedings of the Society for Experimental Biology and Medicine*

the demonstrations cited above of increased cardiac contractile force in dogs. Recent work by Cotten and Stopp (1958) has helped to resolve some of the discrepancies in this area of glycoside action. They found that intravenous administration of ouabain in anesthetized dogs increases left ventricular contractile force concomitantly with decreased atrial pressures, decreased systemic output, increased peripheral resistance and only negligible changes in stroke work. However if

diaphragms with those on isolated ventricle strips of guinea pigs. In low concentrations, the glycoside increases contractile force of the diaphragm when it is stimulated supramaximally either directly or through the phrenic nerve. The responses of the diaphragm are less marked than those of ventricular muscle to the same concentrations of ouabain. Higher concentrations of ouabain cause transient initial augmentation followed by depression of contractile force of the diaphragm. There was in their experiments no evidence of increased excitability of the guinea pig diaphragm to account for the increase in force by ouabain Faust and Saunders reviewed past work on the effects of glycosides on skeletal muscle, pointing out technical factors which probably accounted for conflicting results in the past. It appears that cardiac glycosides augment contractility of all muscle, but that the effect is most striking on cardiac muscle.

The selectivity of glycosides for heart muscle has been attributed to a special affinity of the myocardium for the drugs. Rothlin (1947) concluded that up to 35 times as much of a given dose of a glycoside is taken up by the heart than by other tissues. However Hatcher and Eggleston (1919) were unable to demonstrate digitoxin in rat hearts after parenteral administration although the liver contained demonstrable quantities. With more refined techniques for determination of glycoside content of tissues recent workers have also been unable to show a special affinity of the heart for glycosides (Friedman *et al.*, 1952 Brown *et al* 1956)

The glycoside which is taken up by heart muscle is found primarily in the water soluble and not in the nuclear mitochondrial or microsomal fractions of the cells (St. George *et al.*, 1953 Fischer *et al.*, 1952 Spratt and Okita, 1958)

Present evidence, therefore, does not support the contention that myocardium has a special avidity for cardiac glycosides. It is possible that metabolites of the glycosides are taken up selectively by heart muscle, but no evidence exists for this assumption.

2 *Metabolic Effects*

Numerous attempts have been made to explain in biochemical terms the action of glycosides on myocardial contractility Wollenberger (1949) has summarized this work up to 1949 in an excellent and comprehensive review

It has been recognized generally that cardiac glycosides increase the

mechanical efficiency of the failing heart. Peters and Visscher (1936) found that in the spontaneously failing heart-lung preparation, the administration of a glycoside produced an increase in oxygen consumption and work performance under conditions of constant heart rate and constant external diastolic volume. The per cent increase of mechanical efficiency was greater than that of oxygen consumption indicating an increase in the utilization of energy Since the work of Peters and Visscher nearly all subsequent studies of the effects of glycosides on energy metabolism of heart muscle have substantiated the conclusion that the compounds act primarily by increasing utilization of energy and not by increasing energy production.

Cardiac glycosides have a specific effect on the respiration of myocardium. In low concentrations which approximate those estimated to exist in body tissues after the administration of therapeutic doses, glycosides increase O_2 consumption of isolated slices or segments of myocardium in a variety of animal species such as the dog (Wollenberger 1953) the cat (Finkelstein and Bodansky 1948 Langemann *et al.*, 1953 Lee, 1953a Herrmann *et al.*, 1954) the guinea pig (Wollenberger 1947 Doull *et al* 1951) the mouse (Herrmann 1950) the rat atrium (Ransom and Loomis, 1952) human papillary muscle (Burdette, 1952) and chick embryo hearts (Smith *et al.*, 1954) Herrmann (1950) was unable to find increased O_2 consumption in rat ventricle slices, a finding in keeping with the greater resistance of the rat to glycosides. The concentrations of glycosides which increase O_2 consumption are in the range of 10^{-7} to $10^{-7}M$ In some species, such as the cat (Finkelstein and Bodansky 1948 Langemann *et al.*, 1953) the increased O_2 consumption is maintained for several hours, whereas in the guinea pig (Wollenberger 1947) the initial increase is followed by a decrease.

The stimulant effect of glycosides on heart muscle respiration is dependent on an intact cell structure, since no increase in O consumption is observed in homogenates of guinea pig heart (Wollenberger 1947) or in mitochondrial preparations of cat heart muscle (Langemann, *et al.*, 1953) The significance of this phenomenon is not clear Wollenberger (1953) concluded that it is not due to increased diffusion of substrates into the cell, since glycosides, in concentrations which increase O consumption, do not increase the consumption of C labeled glucose in dog heart slices.

Cardiac glycosides have little or no stimulant action on the respi

simultaneously increases O consumption and oxidation of glucose while reducing glycolysis and the uptake of glucose. Lactate uptake and oxidation are increased concomitantly with the increased O consumption. However the uptake of pyruvate and its oxidation and conversion to lactic acid remain unchanged in the presence of ouabain. Wollenberger postulated that the action of ouabain is centered around pyruvate, increased CO production from glucose and lactate being a result of making more pyruvate available from these precursors to the

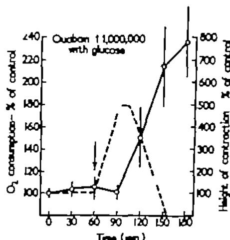


FIG. 3 The effects of ouabain on oxygen consumption and amplitude of contraction of isolated papillary muscle of the cat. The dashed line represents the amplitude of contraction in per cent of control values and the solid line connects the mean O_2 consumptions in per cent of control values. The 99% confidence limits of each mean are depicted by the vertical lines. At the arrow ouabain was added to the bath to make a final concentration of 1 1,000,000 (1.3×10^{-6} *M*) Maximum increase in contractile amplitude was reached in 30 min. Oxygen consumption did not begin to increase until after the maximum increase in contraction was obtained and was accompanied by the development of arrhythmias and the beginning of contracture. Redrawn from Lee (1953a) through the courtesy of the author and *Proceedings of the Society for Experimental Biology and Medicine*.

enzymes which catalyze the oxidative decarboxylation of pyruvate via acetyl coenzyme A and the Krebs cycle. He further speculated that this action involves the lactic dehydrogenase system.

Attempts to demonstrate an action on specific enzyme systems have not been especially successful. Glycosides appear to have no effect on lactic dehydrogenase (Reiter and Barron, 1952) on cytochrome oxidase (Wollenberger 1947 Helmreich, 1950 Doull *et al.* 1951) or on succinic dehydrogenase (Doull *et al* 1951) No effect has been

demonstrated on oxidative phosphorylation (Doull *et al.*, 1951 Langemann *et al.*, 1953, Grisolia 1955 Plaut *et al.*, 1957) or on anaerobic glycolysis (Doull *et al.*, 1951)

Variable results have been obtained concerning the effects of glycosides on adenosinetriphosphatase (ATPase) activity Kimura and DuBois (1947) and Proctor *et al* (1955) reported an inhibition of myocardial ATPase activity by digitoxin. Munchinger (1953) on the other hand reported that glycosides enhance ATPase activity while Reiter and Barron (1952) were unable to demonstrate any effect of ouabain on the ATPase activity of crystalline myosin. The most recent investigators in this area (Read and Kelsey 1957) could also detect no effect of digoxin on ATPase activity of purified actomyosin. They found, however that digoxin blocks the inhibiting effect of myokinase on the ATPase activity of myosin B from dog hearts. They also found that digoxin, at a low concentration, augments myokinase activity in the myokinase controlled reaction ADP → ATP They postulate that digoxin acts by inhibiting ATP hydrolysis and by augmenting ATP resynthesis thus increasing contractility at the expense of relaxation

The influence of glycosides on the concentration of high energy phosphates in the myocardium has been the subject of a number of studies, for the most part yielding equivalent results. The work of Wollenberger (1951) is representative. He found that ouabain and digoxin have little effect on myocardial phosphates in the canine heart lung preparation when administered in doses which produce only a positive inotropic effect. Toxic doses deplete phosphocreatine with no change in ATP These observations, which suggest that non toxic doses of glycosides do not alter the total concentration of high energy phosphates, do not, however preclude a more rapid turnover of phosphate groups which might not be reflected in total concentration. In other experiments, Wollenberger and Karsh (1952) found that dinitrophenol (DNP) markedly reduces the phosphocreatine content of the isolated guinea pig heart without altering ATP concentration. In the DNP pretreated heart, ouabain no longer increases contractile force and reduces by 50% the labile nucleotide phosphate content. Furthermore doses of ouabain which are ordinarily nontoxic are very toxic in the DNP treated heart. Wollenberger and Karsh postulate that these effects represent an acceleration of dephosphorylation by the glycoside. In the DNP poisoned heart with synthesis of ATP blocked, ouabain then depletes the ATP reserves. Although these effects were

observed only under conditions of toxic action, these authors suggest that even under nontoxic conditions the main effect of glycosides is an acceleration of dephosphorylation and that the primary site of attack of the drugs in the myocardial fiber is probably the same during the therapeutic and toxic phases of action. Working with isolated electrically driven left atria of guinea pigs, Furchgott and deGubareff (1956) found no significant differences in phosphate concentrations (ATP ADP, AMP, creatine phosphate and inorganic phosphate) among control (non failing) atria, atria in spontaneous failure and atria restored from failure by k-strophanthin. Excess glycoside decreased both contractile strength and creatine phosphate and ATP These authors concluded that the positive inotropic effect of glycosides is independent of any changes in concentrations of high energy phosphate compounds. They agree, however with Wollenberger (see above) that the inotropic action may be due to a more efficient utilization of energy rich phosphates in the contractile process.

Other investigators have also concluded that the glycosides act by altering the utilization of high energy phosphates. Ellis (1953) using metabolic inhibitors such as dinitrophenol and fluoroacetate on the hypodynamic frog heart, concluded that the positive inotropic action of *k*-strophanthin is not dependent on the functional integrity of oxidative metabolism as long as adequate energy can be derived from anaerobic metabolism. Gruhzit and Farah (1955) found that spontaneous and pentobarbital-induced failure of the heart-lung preparation of the dog is readily reversed by ouabain, whereas the failure produced by sodium azide, sodium cyanide, and dinitrophenol is partially or completely refractory to the effects of ouabain, in proportion to the degree of poisoning by the metabolic inhibitors. Rothlin *et al.* (1955) showed that the failure induced by small doses of DNP in the heart lung preparation is reversed by lanatoside C, although the increased O consumption produced by DNP remains unaltered. Failure resulting from high doses of DNP or by a combination of low doses of DNP and hypoxia was not reversed by the glycoside.

A number of the authors whose work is reviewed above have implied that cardiac glycosides increase cardiac contractile force by increasing the amount of energy from ATP available to the contractile substance. In the light of the recent work of Mommaerts (1955) which casts doubt on the validity of the concept that ATP (or phosphocreatine) is the final energy source for either contraction or relaxa-

tion of muscle, the significance of the actions of glycosides on inorganic and organic phosphates in heart muscle is questionable. Until more is known about the energetics of muscle contraction, particularly of heart muscle, and until more refined methods, such as those used by Mommaerts, are applied to the study of heart muscle, the assumption that glycosides increase cardiac contractile force through actions on the final stage of energy utilization must be considered at best as a tentative hypothesis.

3 *Effects on Contractile Proteins*

The possibility of a direct action of glycosides on contractile proteins has been considered by a few investigators. According to Horváth *et al.* (1949) *g*-strophanthin (ouabain) and digitoxin accelerate the polymerization of actin obtained from heart muscle but not of actin obtained from skeletal muscle. They suggest that glycosides increase myocardial contractility by accelerating the polymerization of *G*-actin to *F* actin. Snellman and Gelotte (1950) obtained similar results. However the significance of this action in relation to the effects of glycosides on myocardial contraction is uncertain, since Wollenberger (1954) demonstrated that both cardio-active and cardio-inactive steroid glycosides augment polymerization of *G*-actin prepared from calf hearts Furthermore, neither the active nor inactive glycosides were found by Wollenberger to affect polymerization of *G*-actin which is prepared from hearts removed from the animals immediately after stunning as compared with actin prepared from hearts which had remained in the animal for some time after death.

Robb and Mallov (1953) observed an increase in ATP-induced contractile activity of actomyosin threads prepared in the presence of ouabain as compared to the contraction of nontreated actomyosin threads. Both skeletal muscle and myocardial actomyosin respond with greater shortening and greater performance of work, but skeletal muscle actomyosin is much less sensitive to the glycoside (Fig 4) Bowen (1951) found that digoxin caused a significantly faster ATP induced shortening of myosin B threads prepared from skeletal muscle as compared to untreated control threads. Edman (1953) found that glycerin extracted fibers of rabbit psoas muscle contract more completely in response to ATP if both ouabain and calcium are in the medium. Neither calcium alone nor ouabain alone had any augmenting effect on the contractions. In this respect, it should be noted that

the results of Robb and Mallov on cardiac actomyosin were obtained in the absence of calcium.

In contrast to these observations, Stutz *et al.* (1954) were unable to detect any effect of lanatoside C on the ATP induced contraction or on the length-resting tension relationships of glycerol extracted fibers from the hearts of dogs which has been given the glycoside for several days before sacrifice. (The heart muscle was additionally exposed to the glycoside during the extraction period of several days.) However

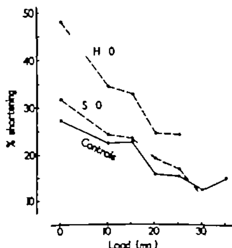


Fig 4 The effect of ouabain on ATP-induced contraction of cardiac actomyosin threads. Abscissa, load in mg.; ordinate, per cent shortening of threads in response to exposure to 0.5% ATP Top curve, average per cent shortening of heart actomyosin fibers (H) in the presence of ouabain (O) Middle curve presents similar averages for skeletal actomyosin (S) plus ouabain (O) Lower curve presents averages for per cent shortening of 185 control fibers, both skeletal and heart actomyosin. Statistical analysis indicates differences are significant. Crystalline ouabain was added to the actomyosin solutions before preparation of threads, making a concentration of 7:1 × 10⁶ (about 10^{-4} *M*) From Robb and Mallov (1953) through courtesy of the authors and *The Journal of Pharmacology and Experimental Therapeutics.*

the glycoside treated fibers did show changes in the action potentials as compared with the normal controls. It is not clear why the contraction of glycerol extracted myocardial fibers is unaffected by glycosides when myocardial actomyosin threads appear to contract more forcefully (Robb and Mallov 1953) This discrepancy may lie in the differences of technique or in the doses of the glycosides which were used by these two groups of workers.

The viscosity of skeletal muscle actomyosin solutions has been orted to be decreased by several glycosides (Waser and Volkart, 4) This effect is abolished by ethanol or methanol. The viscosity of osin was unchanged by glycosides.

Relationship between Glycosides and Ions

Calcium resembles cardiac glycosides in its actions on heart muscle reasing concentration of calcium augments cardiac contractile ce, causing arrhythmias in high doses. Calcium also increases the consumption and mechanical efficiency of the failing heart lung paration (Peters and Visscher 1936) An additive or synergistic ationship between calcium and glycosides exists in terms of cardiac ection. Reduction of the concentration of calcium in the bath of the lated frog heart results in diminished contractile activity and reced effectiveness of the glycosides (Clark, 1912 Salter *et al.*, 1949b) owever the hypodynamic contractions caused by calcium deficiency e restored to normal in the frog heart (Ransom, 1917 Salter *et al* 49a) and in the cat papillary muscle (Cattell and Gold 1938) by the ldition of a glycoside. Salter *et al* (1949a) have shown that the per ntile contractile response of the frog heart to a given dose of ouabain creases with increasing concentrations of calcium. Other examples of is interrelationship between calcium and glycosides include their fects on cardiac rhythm (Gold and Edwards, 1927–1928) the in ence of calcium on the fatal dose of glycosides (Lieberman, 1933 loyd, 1928) the increased O_2 consumption of heart muscle in re onse to glycosides (Section II C, 2) and the rhythmic activity of nbryonic chick hearts (Paff 1940)

The precise mechanism by which calcium and glycosides interact unknown. Burridge (1916) suggested that the glycosides act by in reasing the responsiveness of the heart to calcium. Salter *et al* (1949a) und that each molecule of ouabain can compensate for the loss of a iven number of molecules of calcium in the frog heart, a finding in ipport of Burridge's suggestion. Friedman and Bine (1948) found that lycosides exert their typical effects on isolated embryonic duck hearts a calcium-free medium. Since calcium was undoubtedly present in he myocardial cells, this observation does not rule out the possibility hat the effect of glycosides is mediated through increasing the sensi ivity of the cells to calcium. Also the fact that actin contains calcium s an integral part of the molecule and that polymerization of G-actin

to *F*-actin depends on an optimal ratio between calcium and potassium suggests an intimate relationship between the glycosides and calcium, if one assumes that glycosides act directly on contractile proteins (Sections II C 3 and VI B 2)

Potassium appears to modify the action of calcium on the contractile response of the frog heart to cardiac glycosides Salter and Runels (1951) found a quantitative relationship between the relative concentrations of calcium and potassium in the medium and the contractile response to ouabain. For any given concentration of calcium, changes in the concentration of potassium in either direction from an optimal value of 4 8 m*M* decrease the response to ouabain. In contrast, Garb and Venturi (1954) found that the effect of ouabain on the force of cat papillary muscle is unchanged by alterations in potassium concentration between 3.5 and 8.5 m*M* although the onset of arrhythmias is delayed or prevented at the higher potassium concentrations.

Cardiac glycosides have been shown to reduce the influx of potassium into myocardial cells (Hajdu, 1953, Rayner and Weatherall, 1957) and human erythrocytes (Schatzmann, 1953 Kahn and Acheson, 1955) Hajdu suggested that the positive inotropic action of glycosides is a result of a reduction of intracellular potassium content secondary to diminished re-entry of potassium into the myocardial cells during relaxation producing a more optimal intracellular environment for contraction of the contractile proteins. Hajdu s hypothesis is based on the effects of potassium and glycosides on the abolition of the staircase phenomenon of the isolated ventricle of the frog (see section VI, B 1) The mechanism of inhibition of potassium re-entry is unknown, although it has been suggested that glycosides, by affecting cholinesterase, alter cellular permeability to ions (Govier *et al* 1953 Holland *et al*., 1954) According to this concept, the permeability of the cell membrane is controlled by acetylcholine. Thus, cholinesterase, by governing the amount of acetylcholine present, regulates the rate of ion movements across the membrane. Cardiac glycosides are believed to compete with acetylcholine for the active sites on cholinesterase and thus to affect intracellular potassium and sodium content.

Most early work indicated that the potassium content of cat and dog hearts *in vivo* is unaffected by small doses of cardiac glycosides, although toxic doses cause a decrease (Calhoun and Harrison, 1931 Wedd 1939 Boyer and Poindexter 1940 Sherrod 1947) In the canine heart-lung preparation, however Wood and Moe (1940)

found a correlation between the dose of a glycoside and the rate of increase in serum potassium. The rate of increase in serum potassium was also correlated with the extent of improvement in external cardiac efficiency. The failure of the former groups of investigators to find a significant decrease in myocardial potassium content *in vivo* may be related to the use of chemical methods for determining the concentration of potassium. The use of the more sensitive flame photometric analysis has shown that small doses of cardiac glycosides do cause a significant loss of intracellular potassium as evidenced by the development of a negative coronary arteriovenous (A-V) potassium difference (Hellems *et al.*, 1955; Bay and Bay, 1956). Associated with the loss of potassium, there is increased uptake of sodium by the heart. These effects are similar to those occurring in the frog heart. Gonlubol *et al.* (1956) could not confirm these findings in patients with cardiac disease, but, as they pointed out, the failure to find a significant A-V potassium difference after small doses of glycosides may be due to a slower rate of potassium loss from the human heart as compared with the dog heart. An alternate possibility is that the diseased human heart may have a lowered potassium content and therapeutic doses of glycosides do not reduce it further. This would imply that the increased contractility provided by glycosides is not directly related to alterations in intracellular environment. This possibility is supported by the observations (Vick, 1958) that angelicalactone and glycosides cause isolated, hypodynamic guinea pig hearts to lose similar quantities of potassium, although the former compound depresses contractility while glycosides stimulate the heart.

D. Summary

The contraction of heart muscle develops more forcefully, rapidly and efficiently in the presence of small amounts of cardiac glycosides. This effect occurs in both failing and nonfailing hearts. While the mode of action is unknown, present evidence points to at least three possible sites of action: (1) the ultimate steps in the transfer of energy to the contractile proteins, (2) the contractile proteins, and (3) the ionic transport mechanism either at the cell membrane or within the cell.

The significance of much of the work on basic mechanisms of glycoside action, however suggestive, can not be adequately assessed at present. The incomplete understanding of the contractile process is

partly responsible. In addition, many investigators have not attempted to correlate their observations on changes in certain noncontractile cell functions (such as O_2 consumption, phosphate concentration etc.) with changes in contraction itself. Increased emphasis on such correlations would help clarify the actions of these drugs, such as was done by Lee (1953a) who simultaneously measured changes of contractile force and O_2 consumption in response to ouabain. Also neglected has been the use of glycosides which lack effects on contractility in experiments on energy metabolism and contractile proteins, such as was done in the work of Wollenberger (1954) on the polymerization of *G*-actin

III. SYMPATHOMIMETIC AMINES

Most sympathomimetic amines increase the contractility of cardiac muscle, an action which is qualitatively similar to that produced by stimulation of the cardiac sympathetic nerves. The first description of sympathomimetic activity was that of Oliver and Schafer (1895) who observed a marked rise in blood pressure of the dog after intravenous injection of an extract of the suprarenal gland Following the isolation and identification of epinephrine from the adrenal gland by Abel and Crawford (1897) and Takamine (1901) and its synthesis by Stolz (1904) and Dakin (1905) some 57 amines were synthesized and studied by Barger and Dale (1910) Since many of the latter amines produced epinephrine like effects, they proposed the name sympathomimetic for this class of drugs. The latter investigation paved the way for further development of a variety of sympathomimetic amines, many of which are currently employed in therapeutics as cardiac stimulants, vasopressor agents, bronchodilators, local vasoconstrictors, nasal decongestants, and central nervous system stimulants.

A. SOURCES AND CHEMISTRY

With the exception of epinephrine and norepinephrine, which are found in animal tissues, and of ephedrine, which is obtained from a plant, virtually all of the sympathomimetic amines are of synthetic origin. Even those which occur in nature are now made synthetically

Sympathomimetic activity is found in numerous amines, including simple aliphatic and alicyclic amines and aromatic alkylamines. Most of the important compounds are derivatives of phenylalkylamines. Epinephrine, for instance, can be considered as a substituted derivative of β-phenylethylamine.

A large literature exists concerning the relationships between chemical structure and pharmacological activity in this series of compounds. (Hartung 1945 Beyer 1946 Lands, 1949 also see Goodman and Gilman (1954) for a description of these relationships and for additional references) However while certain relationships are prominent, it is not possible at the moment to define precisely the structural requirements for optimal activity, particularly in regard to effects on the heart.

B Effects on Myocardial Contractility

The increase in contractile force evoked by sympathomimetic amines represents a direct action upon myocardial cells. The intensity is a function of the dose employed (Fig 5) Most amines also increase the

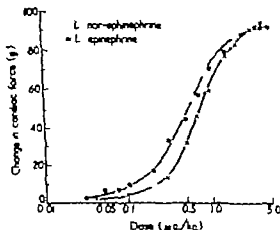


Fig. 5 Representative dose-response curves of the effects of *l*-epinephrine and *l* norepinephrine on the force of ventricular contraction of anesthetized, vagotomized, open-chest dogs Each change in contractile force was calculated from the control immediately preceding the injection of the drugs. Similar curves were obtained in unanesthetized, unpremedicated dogs. From Cotten and Pincus (1955) through the courtesy of *The Journal of Pharmacology and Experimental Therapeutics*

heart rate of spontaneously beating hearts, but the increased force is not dependent upon increased rate (Lee, 1953b)

The sympathomimetic amines vary greatly in the magnitude and duration of their actions on the heart. Some, like epinephrine, produce marked, transient increases in contractile force while others, such as ephedrine cause more prolonged stimulation. Still others, such as methoxamine, are practically devoid of cardiac effects. The peripheral

vascular actions of these drugs are not necessarily correlated with their effects upon contractile force, e.g. isopropylnorepinephrine, a vasodilator amine, markedly augments contractile force while methoxamine, a vasoconstrictor, has no stimulant effect on contractile force.

Epinephrine, norepinephrine, and isopropylnorepinephrine are typical potent cardiac stimulants with short durations of action. Their relative potencies vary from one animal species to another and even the auricular and ventricular muscle of the same species vary in responsiveness to equimolar doses. Table I, which contrasts the effects of these amines on the amplitude of contraction of isolated auricles of the cat, guinea pig, rabbit, and rat, shows that epinephrine is generally more potent than norepinephrine, while isopropylnorepinephrine is the most potent of all (Garb *et al.*, 1956). In contrast, epinephrine and

Table I

Effect of Three Sympathomimetic Amines on Amplitude of Contraction of Spontaneously Beating Isolated Auricles of Several Species[a]

Drug	Concentration producing 40% increase (µg./ml.)	Relative potency	
		Rat equals I	Nor epinephrine equals I
Rat			
Norepinephrine	$2 \times 10^{-[illegible]}$	I	I
Epinephrine	$1 \times 10^{-[illegible]}$	I	20
Isopropylnorepinephrine	$5 \times 10^{-[illegible]}$	I	400,000
Guinea-pig			
Norepinephrine	3×10^{-4}	7	I
Epinephrine	1×10^{-4}	10	30
Isopropylnorepinephrine	$2 \times 10^{-[illegible]}$	I/400	150
Rabbit			
Norepinephrine[b]		I/30	I
Epinephrine[b]		I/40	16
Isopropylnorepinephrine	7×10^{-4}	I/13,000	1,000
Cat			
Norepinephrine	$7 \times 10^{-}$	3	I
Epinephrine	$3 \times 10^{-}$	I/3	2
Isopropylnorepinephrine	5×10^{-4}	I/10,000	14

[a] Data from Garb *et al.* (1956). Courtesy of the authors and *The American Journal of Physiology*.

[b] Calculation based on less than a 40% increase in force.

norepinephrine are approximately equipotent on the spontaneously beating rabbit heart (Hilton, 1955) and on ventricular contractile force in both conscious and anesthetized dogs (Zanetti and Opdyke, 1953 Cotten and Pincus, 1955 West and Rushmer 1957) (see Fig 5) In intact dogs, isopropylnorepinephrine is approximately four times as potent as epinephrine and norepinephrine (Cotten and Moran, unpublished observations)

Epinephrine and norepinephrine also increase the velocity of contraction (Wiggers, 1927b Cotten and Bay, 1956) and the distensibility of ventricular muscle (Rushmer 1955 p 101) The relaxation phase of the cardiac cycle is shortened, an effect readily demonstrated when heart rate and arterial pressure are kept constant (Wiggers, 1927b Opdyke, 1952) Increasing the rate of relaxation of ventricular muscle increases the time available for cardiac filling during diastole, while increased distensibility permits greater filling of the ventricular chambers at the same filling pressure. These factors combined with an increased contractile force and venous return, allow more complete filling and emptying of the heart with each beat.

Other amines which are capable of increasing contractile force of heart muscle to approximately the same extent as epinephrine and norepinephrine differ from these two amines in that they are less potent and have a longer duration of action. Ephedrine, which is a typical example, has been shown to increase contractile force of the dog heart *in situ* (Chen and Schmidt, 1924 Walton and Brodie, 1949 Goldberg *et al.*, 1953) and of the papillary muscle of the cat (Krop 1944) A number of other amines have been shown to have a similar type action on the heart (Crismon and Tainter 1938 Goldberg *et al.*, 1953)

Other vasoconstrictor amines, such as methoxamine and phenylephrine, evoke little or no increase in contractile force in doses causing substantial rises in blood pressure. Methoxamine has no positive inotropic action on the isolated rabbit heart (Melville and Lu 1952) or on the dog heart *in situ* (Alexander 1952 Goldberg *et al* 1953) In the canine heart-lung preparation, the administration of as much as 40 mg of methoxamine has no effect on cardiac activity (Cotten, unpublished observations) Like methoxamine phenylephrine has little effect on contractility in doses which produce a 50-mm. Hg rise in the blood pressure of dogs (Goldberg *et al.*, 1953) Myocardial force in dogs subjected to hemorrhagic shock also does not appear to be influenced by phenylephrine (Opdyke, 1944) the drug produces

cardiac dilation in man, suggesting the absence of myocardial stimulation (Keys and Violante, 1942) However the injection of ten times the usual pressor dose in anesthetized dogs markedly increases contractile force and blood pressure indicating that phenylephrine, unlike methoxamine is inherently capable of stimulating the heart (Cotten, unpublished observations) In this connection, phenylephrine has also been shown to increase cardiac output and heart rate in the canine heart-lung preparation (Crismon and Tainter 1938) Apparently phenylephrine has potent vasoconstrictor properties in doses which are considerably smaller than those required to produce cardiac stimulation. In contrast, contractile force and blood pressure increase approximately in parallel after administration of graded doses of epinephrine and norepinephrine (Cotten and Pincus, 1955)

C. Evidence for Myocardial Stimulation in Intact Animals and Man

Attempts to determine the effects of sympathomimetic amines on the contractile force of cardiac muscle in intact animals or human beings by hemodynamic analysis has often led to confusion regarding the fundamental behavior of the drugs on the heart. For example, Goldenberg *et al.* (1948) found that intravenous infusion of small doses of epinephrine in human subjects increases heart rate and cardiac output with little change in mean arterial pressure, whereas comparable doses of norepinephrine decrease heart rate and raise mean arterial pressure leaving cardiac output unchanged. These data would suggest that norepinephrine, in contrast to epinephrine, has no cardiac stimulant action in man. However calculations from their data show that both drugs increase left ventricular stroke work to approximately the same extent. Since there is no evidence that the heart dilates to perform the increased stroke work after norepinephrine administration, the inadequacy of measurements of cardiac output alone to evaluate the positive inotropic actions of drugs becomes apparent.

An example of the fact that even stroke work is not always a reliable measure of the actions of drugs on myocardial contractility is seen in the effect of large doses of isopropylnorepinephrine on anesthetized dogs (Cotten unpublished experiments) where the amine causes a marked increase in left ventricular force with a decrease in calculated left ventricular stroke work. The latter is the result of a combination of reduced stroke volume secondary to cardio-acceleration and decreased

arterial pressure. Calculated left ventricular stroke work is not, however always decreased by isopropylnorepinephrine but depends upon the magnitude of the changes in hemodynamic factors, which are in turn dependent upon the dose employed For example, in conscious human beings, the intravenous infusion of small doses of the drug which increase heart rate only slightly and which do not change mean blood pressure, substantially increases left ventricular stroke work and cardiac output (Dodge and Murdaugh, 1957) These examples emphasize the importance of complete hemodynamic analysis of cardiovascular drugs, especially in conscious subjects where compensatory mechanisms modify cardiac actions. (For more detailed discussion see Sarnoff *et al* 1954 Rushmer and West, 1957 Cotten and Stopp 1958)

D Mechanism of Action

Although most sympathomimetic amines have marked actions upon myocardial contractility only epinephrine has been extensively studied with respect to its cellular effects. These will be discussed using the scheme employed in considering the cardiac glycosides, namely changes in energy sources for myocardial contraction, effects on contractile proteins, and effects on the ionic content of heart muscle.

1 *Changes in Energy Sources for Myocardial Contraction*

Epinephrine reduces the glycogen content of cardiac muscle *in vivo* (Chang 1936–1937 Mulder *et al* 1952) and *in vitro* (Cruickshank, 1913 Patterson and Starling 1913) The increased glycogenolysis appears to be due to augmentation of phosphorylase activity by epinephrine, following the same general pattern as is seen in skeletal muscle and liver (see Ellis, 1956 1959 for references) According to Sutherland and Rall, epinephrine activates phosphorylase indirectly by increasing the accumulation of adenosine-3 5 phosphoric acid (3,5-AMP) from ATP 3,5-AMP is then involved in the conversion of phosphorylase *b* (inactive) to phosphorylase *a* (active) (Rall and Sutherland 1958, 1959 Sutherland and Rall, 1958) Activation of phosphorylase of heart muscle can be produced by sympathomimetic amines which stimulate contraction (Hess and Haugaard, 1958) but not by those such as methoxamine, which lack myocardial stimulant properties (Haugaard *et al* 1959a Mayer and Moran, 1959 and unpublished observations) Dichloroisoproterenol (DCI) a specific

cardiac adrenergic blocking drug (see Section III D-4) has been found to antagonize both the activation of myocardial phosphorylase and the augmented contractile force produced by epinephrine and cardiac sympathetic nerve stimulation in open chest dogs (Mayer and Moran, 1959 and unpublished observations) Among cardiac stimulants only sympathomimetic amines appear to be capable of activating phosphorylase in dog hearts calcium chloride (except in excessive doses) theophylline and ouabain increased contractile force but produced no activation of phosphorylase (Mayer and Moran, 1959 and unpublished observations) Hess and Haugaard (1958) however have described increased phosphorylase *a* activity in isolated perfused rat hearts in response to aminophylline. It should be noted that aminophylline is a complex of theophylline and ethylene diamine, the latter having positive inotropic actions (Hardman *et al.* 1959) possibly sympathomimetic in nature.

The nature of the relationship of activation of myocardial phosphorylase to the augmented contractile force produced by sympathetic stimuli has not been established conclusively Haugaard *et al* (1959b) reported a positive correlation between the magnitudes of increase contractile force and increased phosphorylase *a* activity in isolated perfused hearts of rats in response to varying doses of catechol amines. However it is not possible to conclude whether the epinephrine-induced activation of phosphorylase in myocardium is causally or coincidentally related to the epinephrine induced stimulation of the contractile mechanism.

The uptake of glucose by isolated rabbit hearts (Loewi and Weselko, 1914 Wilenko 1913) and by isolated rat diaphragm (Cohen 1947) obtained from epinephrine treated animals is depressed but this effect is not observed if epinephrine is added *in vitro* In contrast, the uptake of glucose is enhanced in the canine heart-lung preparation (Patterson and Starling 1913 Evans and Ogawa, 1914) an effect probably due to the increased work performed by the heart after epinephrine injection. The uptake of pyruvate is unchanged by epinephrine in both the isolated, perfused heart and in the heart-lung preparation of the dog (Braun-Menendez *et al* 1939)

Oxygen consumption of resting heart muscle is unaffected by epinephrine (Century 1954 Herrmann *et al.*, 1954) but is markedly increased in working heart muscle (Gollwitzer Meier and Krüger 1938 Gremels, 1940 Lee, 1953b) As in resting cardiac muscle, O

uptake by the isolated rat diaphragm is not affected by epinephrine, neither when injected into the animal prior to removal of the diaphragm nor when added *in vitro* (Walaas and Walaas, 1950) Both epinephrine and norepinephrine cause a simultaneous, marked increase in O_2 uptake and contractile force of the cat papillary muscle (Fig 6) with no change in heart rate (Lee, 1953b) Others have also

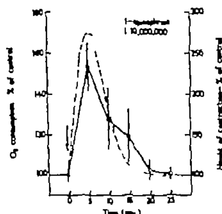


FIG. 6. The effects of *l*-epinephrine on oxygen consumption and amplitude of contraction of isolated papillary muscle of the cat. The dashed line represents the amplitude of contraction in per cent of control values and the solid line connects the mean O_2 consumptions in per cent of control values. The 99% confidence limits of each mean are depicted by the vertical lines. At the arrow *l*-epinephrine was added to the bath to make a final concentration of 1:10,000,000 (about 5×10^{-9} *M*) Note the simultaneous increase, followed by simultaneous decrease, in both O_2 consumption and contraction amplitude. Maximum effect was obtained in 5 min. with return to control values within 30 min. Redrawn from Lee (1953b) through the courtesy of the author and *The Journal of Pharmacology and Experimental Therapeutics*.

noted that increased heart rate is not essential for the increased O_2 consumption after epinephrine administration (Kramer *et al* 1950) although Gollwitzer Meier and Kroetz (1939) found that there was less O uptake when the cardiac actions of epinephrine were modified by neural reflexes than when they were unopposed The fact that the uptake of neither glucose nor O_2 is affected in resting muscle, but is markedly increased in working muscle, suggests that the augmenting effect of epinephrine on these functions is the result of the increased heart work and not an effect of epinephrine *per se* When the working heart is stimulated by epinephrine the increase in phosphorylase activity may play an important rôle in supplying glucose to the heart muscle.

The substantial increases in both O_2 uptake and contractile force of working heart muscle suggest that myocardial efficiency is not increased by epinephrine but this problem remains unresolved (Ellis, 1956). In contrast, cardiac glycosides definitely increase the efficiency of contraction of failing heart muscle (Section II, C, 2) The striking difference between the effects of ouabain and epinephrine on O_2 consumption and contractile force of isolated myocardium may be seen by comparing Fig 6 with Fig 3

The energy required for increased contractility of the frog heart due to epinephrine administration can be provided either from anaerobic or aerobic sources. For example, Ellis (1953) found that epinephrine increases contractile force of the frog heart under nitrogen, provided that glucose is present as an anaerobic source of energy In addition, blockade of the Krebs tricarboxylic acid cycle with fluoroacetate does not prevent the stimulant action of epinephrine on either the hypodynamic frog heart (Ellis, 1953) or on hypodynamic cat and rabbit papillary muscles (Chenoweth and Pengsritong 1950) Blockade of anaerobic metabolism in the frog heart with sodium iodoacetate also does not inhibit the response to epinephrine, indicating that sufficient energy is available from other sources (Ellis 1953) the energy for increased contractility apparently was not derived from glycogenolysis since these hypodynamic hearts were considered to be depleted of glycogen.

The contractile response to epinephrine in frog hearts is dependent upon the presence of ATP since the response is completely prevented by small concentrations of dinitrophenol (Ellis, 1953) In this connection Century (1954) observed that epinephrine does not influence the rate of breakdown of ATP either in homogenates or in slices of rat hearts. However when the work of the dog heart *in vivo* was augmented by an infusion of epinephrine, the concentration of ATP was decreased (Mulder *et al.*, 1952) These observations again suggest that increased energy production and utilization after administration of epinephrine *in vivo* is the result, and not the cause, of increased cardiac work.

2 *Effects on Contractile Proteins*

The only paper which has come to the attention of the authors concerning the effects of epinephrine on contractile proteins is that of Straub *et al* (1948) which demonstrates that epinephrine enhances the rate of polymerization of actin from globular to fibrous forms. The rate

of this reaction depends upon the ratio of potassium and calcium present in the medium, being greatest when the K/Ca ratio is near the physiological optimum.

3 *Effects on Ionic Content of Heart Muscle*

Robertson and Peyser (1951) reported that large intravenous doses of epinephrine (50 µg per kilogram) or norepinephrine (10 µg per kilogram) have no significant effect upon intracellular potassium or sodium content of the cat heart. Bay and Bay (1956) also concluded that myocardial potassium content was unchanged by norepinephrine in the normal dog since there was no change in the coronary arteriovenous potassium difference even though both arterial and venous potassium concentrations were increased. In contrast, Hajdu (1957) found that the addition of epinephrine to the isolated frog heart abolished the staircase phenomenon, an effect presumably due to a reduction in intracellular potassium content (Section VI B 1) It has also been shown that epinephrine, norepinephrine isoproterenol and aminophylline cause a transient net loss of potassium (K^{39}) from isolated, perfused rabbit hearts as well as an increased rate of K^{42} turnover (Melville and Korol, 1958) These investigators suggested that the observed potassium disequilibrium might cause changes in myocardial permeability which conceivably act as "trigger mechanisms" for the increased contractility evoked by sympathomimetic amines. However the exact rôle of changes in intracellular potassium in initiating or maintaining the positive inotropic effects of epinephrine and related agents remains obscure even to the extent that it is still uncertain whether the changes in potassium represent causal events or merely secondary alterations.

4 *Selective Blockade of Sympathomimetic Drugs on the Heart*

The effects of sympathomimetic compounds on contractile force of mammalian myocardium can be differentiated from the stimulant actions of other types of drugs by the use of a new type of adrenergic blocking agent, dichloroisoproterenol. This compound β-hydroxy N isopropyl 3 4-dichlorophenethylamine hydrochloride (DCI) antagonizes the positive inotropic actions of sympathomimetic amines and of cardiac sympathetic nerve stimulation in the open-chest dog and of amines in the isolated perfused heart of the rabbit, while not altering the cardiac stimulant effect of glycosides, calcium chloride or theo-

phylline (Moran and Perkins, 1958) Selective cardiac adrenergic blockade by DCI has been confirmed on the isolated right ventricle strip of the rat (Moran unpublished observations) and on the isolated atrium of the rabbit (Moran, unpublished observations Furchgott, 1959) The observation that DCI blocks the cardiac responses to sympathetic stimuli supports the concept of Ahlquist (1948) that cardiac adrenotropic receptors are related to adrenergic inhibitory receptors in other tissues. Ahlquist classified these as *beta* receptors in contrast to *alpha* receptors which subserve most adrenergic excitatory functions, such as vasoconstriction Moran and Perkins (1958) have proposed that adrenergic blocking drugs be designated as either *alpha* or *beta* depending on the type of receptor blocked. Thus, DCI, a *beta* adrenergic blocking drug antagonizes the cardiac responses of sympathomimetic amines while the older adrenergic blocking drugs such as phenoxybenzamine and phentolamine (*alpha* blocking drugs) have no specific myocardial adrenergic blocking properties (Moran and Perkins, unpublished observations) Cotten *et al* (1957) have reported that the latter two blocking agents, phentolamine and phenoxybenzamine, produced cardiac sympathetic blockade. It is now recognized that these agents may cause a non-specific depression of myocardial contractile responses and possess no significant specific cardiac blocking actions (Moran and Perkins, unpublished observations) In addition, Mayer and Moran (1959 and unpublished observations) have shown that DCI but not phenoxybenzamine, inhibits the epinephrine induced activation of phosphorylase in the dog heart (see III D-1)

Recent experiments (Cotten and Cooper unpublished observations) have shown that norepinephrine, epinephrine, ephedrine, methamphetamine, aramine, theophylline and ethylene diamine all decrease ventricular contractile force of dogs after digitalization at body temperatures of between 28-30° C. This is in distinct contrast to the increased contractility induced by these drugs at normal body temperature and also during hypothermia before administration of cardiac glycosides. Digitalization at normal body temperature does not affect responses of the heart to these amines upon cooling to 28-30° C. the responses are then reduced but are not reversed. Although digitalization during hypothermia consistently causes reversal of the positive inotropic effects of norepinephrine and other drugs in dogs, this phenomenon is not observed in either cats or rabbits The physiological and/or biochemical bases for these observations remain to be elucidated.

While these observations do not suggest the fundamental mechanism of action of sympathomimetic drugs, they do indicate that their mechanisms differ in some ways from those of other drugs, such as glycosides, in terms of their effects on myocardial contractility

E. Summary

Sympathomimetic amines, like the cardiac glycosides, directly increase the force of contraction and its rate of development, as well as accelerating the relaxation of cardiac muscle. Unlike the glycosides, the amines apparently do not increase the mechanical efficiency of the heart. Because of limited data, even less can be said about the mechanism of action than about that of the glycosides. However on the basis of studies with selective blocking agents, the myocardial stimulant action of sympathomimetic amines can be distinguished from that of other drugs, suggesting a specificity of effect. Whether the site of action of the amines is intracellular or at the cell membrane is unknown. It appears that the increased metabolic activity which sympathomimetic amines produce in myocardium is secondary to and not a result of, the increased contractile activity

IV ERYTHROPHLEUM ALKALOIDS

The Erythrophleum alkaloids, obtained from the bark of the African trees, *Erythrophleum guineense* and *E. couminga*, have actions resembling those of cardiac glycosides. The alkaloids are phenanthrene-like tricyclic acids esterified with dimethylaminoethanol or methylaminoethanol The chemistry of these alkaloids has been reviewed by Dalma (1954)

Eight or more alkaloids have been isolated and studied chemically but only five have been studied for cardiac effects. These are cassaine, cassaidine, coumingine, coumingidine, and erythrophleine, which are structurally similar in that selenium dehydrogenation of their acids yields 1 7 8-trimethylphenanthrene. All of the alkaloids have the same type of activity on the heart, but vary in potency and in the ratio of the dose producing inotropic actions to that producing cardiac arrhythmias.

A. Effects on Myocardial Contraction

Although systolic contracture of the frog heart was described as early as 1875 the first definitive description of positive inotropic

actions was by Maling and Krayer in 1946 These authors found that cassaine, cassaidine, coumingine, and erythrophleine reverse the pentobarbital induced failure of the canine heart-lung preparation, in addition to causing characteristic glycoside like arrhythmias. Coumingine also increases the contractile force of the hypodynamic papillary muscle of the cat (White and Salter, 1946) In dogs with intact circulatory systems, these alkaloids increase cardiac contractile force (Goldberg and Walton, 1952 Cotten *et al.*, 1952) In contrast to the effects of ouabain, the erythrophleum alkaloids have a more rapid onset of action and a shorter period of ectopic activity at high doses.

The ratio of positive inotropic dose to minimal irregularity dose in the heart lung preparation decreases in the order cassaine > cassaidine > erythrophleine > coumingine (Maling and Krayer 1946) In the intact dog the ratio is greater for cassaine and cassaidine than for ouabain, while coumingine produces arrhythmias at approximately the same dose which causes increased contractile force (Goldberg and Walton, 1952 Cotten *et al.* 1952)

The tricyclic acids obtained from hydrolysis of the ester alkaloids have no cardiac action (Maling and Krayer 1946 Uhle *et al.*, 1956) The dimethylaminoethanol moiety of the esters, however does elicit a positive inotropic effect in the canine heart-lung preparation (Krayer *et al* 1946) and in the intact dog (Cotten and Moran, unpublished observations) This effect more closely resembles that of epinephrine than that of the erythrophleum alkaloids or of the cardiac glycosides. Esterification of dimethylaminoethanol with the dibasic succinic, glutaric, adipic, and pimelic acids results in a five to tenfold increase in potency on a molar basis (Uhle *et al.*, 1956) but the type of action is still more closely related to that of epinephrine. Thus the characteristic action of erythrophleum alkaloids on the heart seems to depend upon the ester linkage between an amino alcohol and cassaic acid or its congeners.

B MECHANISM OF ACTION

There are few data available concerning the manner in which erythrophleum alkaloids increase myocardial contractility Wollenberger (1949) has demonstrated that coumingine, in slices but not in homogenates of guinea pig hearts, produces biphasic changes in O consumption similar to those produced by cardiac glycosides. A further similarity between erythrophleum alkaloids and cardiac glycosides is

seen in the abolition of the staircase phenomenon of the isolated frog heart, where coumingine has an activity approximating that of strophanthin (Hajdu, 1957)

C. Summary

Erythrophleum alkaloids, phenanthrene-like compounds obtained from African trees, affect myocardial contraction in a manner similar to that of the cardiac glycosides. Virtually nothing is known about the basic mechanisms of action except that they appear to resemble the glycosides on their effects on myocardial respiration and on their effects on the staircase phenomenon of the frog heart. It is unfortunate that this interesting and potentially useful class of drugs has received so little study

V VERATRUM ALKALOIDS

Veratrum alkaloids are organic bases of plant origin which have complex pharmacological actions involving the cardiovascular system, the nervous system, and the skeletal muscles. In the form of crude extracts, they have been used in the past in the treatment of many unrelated conditions. However within recent years, as a result of the isolation and pharmacological description of pure alkaloids, their clinical use has been restricted to the therapy of hypertensive states

A. Sources and Chemistry

Veratrum alkaloids are obtained from various genera of the family *Liliaceae* such as *Veratrum album* *Veratrum viride* *Schoenocaulon officinale* (*Veratrum sabadilla*) and *Zygadenus venenosus* Two main classes of alkaloids have been designated on the basis of chemical types. These are (1) C^{27} bases with low oxygen content occurring in nature as free bases or in glycosidic combination. Of these, some are typical steroidal alkaloids (rubijervine) while others are secondary amine variants of steroidal alkaloids (veratramine and jervine) (2) Highly oxygenated C^{27} tertiary alkamines, which occur in nature as esters containing acyl groups derived from simple organic acids. The fundamental ring structure of this group resembles that of the secondary amine alkaloids, such as veratramine. The glycosidic alkaloids and corresponding alkamines are qualitatively similar as are the tertiary amine ester alkaloids and their corresponding alkamines. However the ester alkaloids are many times more potent than their alkamines. The division into two chemical classes is reflected in distinct differences in pharma

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cological actions. For reference to the chemistry of veratrum alkaloids see the paper by Barton *et al* (1954) which proposes a new structure for veracevine, the alkamine of veratridine, and to the review by Prelog and Jeger (1953)

Related pharmacologically to the tertiary amine ester alkaloids, although differing chemically is acetylandromedol (andromedotoxin) a polycyclic, neutral, crystalline compound obtained from the leaves of *Rhododendron maximum* and other plants of the Ericaceae family The structure of acetylandromedol is as yet unknown, although a triterpene ring system has been proposed for related compounds For a recent description of the chemistry see Tallent *et al* (1957)

B Effects on Myocardial Contractility

The tertiary amine ester alkaloids of veratrum and acetylandromedol reflexly produce in intact animals bradycardia, decreased blood pressure, and respiratory depression, as a result of stimulation of sensory receptors in the heart, carotid sinuses, aortic arch, and the lungs In addition, high doses of these compounds increase cardiac contractile force, produce cardiac arrhythmias, and raise blood pressure, effects which are not prominent in intact animals because of the predominance of the reflex vasodepression and cardiac slowing [See Krayer (1954) for a description of the actions of veratrum alkaloids and Moran *et al.* (1954) for a description of the effects of acetylandromedol.]

The tertiary amine ester alkaloids, particularly veratridine, produce unique effects on nerve and skeletal muscle. The action on muscle, termed the "veratrine response" is composed of a tetanic prolongation of muscle contraction following a single twitch. This is accompanied by a series of repetitive electrical discharges superimposed on the negative after potential (Witt and Swaine, 1957)

The secondary amine alkaloids and their glycosides differ from the tertiary amine ester alkaloids in that they lack the reflex circulatory and respiratory actions, the cardiac inotropic and arrhythmic effects, and the veratrinic action on skeletal muscle. Instead they possess antiveratrinic actions on skeletal muscle and antagonize the cardioacceleration produced by adrenergic stimuli (Krayer 1954)

1 *Direct Effects on Contractility*

The tertiary amine ester alkaloids and their alkamines augment myocardial contractility both directly and indirectly Early studies

dealt mainly with descriptions of the actions of veratrine on isolated hearts. (Veratrine is a mixture of alkaloids from *Schoenocaulon officinale* principally veratridine and cevadine.) For a comprehensive review of early work on these alkaloids, see Krayer and Acheson (1946)

Modern investigations of the actions of veratrum alkaloids on myocardial contractility stem from the demonstration by Krayer and Mendez (1942) that veratrine reverses the spontaneous or pentobarbital induced failure of the canine heart lung preparation. Subsequently similar effects were described for various pure alkaloids such as veratridine and cevadine (Moe and Krayer 1943) protoveratrine (Krayer *et al.*, 1944), and several new alkaloids isolated from *Zygadenus venenosus* (Krayer *et al.*, 1953) Protoveratrine, in comparison with veratridine does not completely reverse the failure. The tertiary amine ester alkaloids are singularly potent—as little as 5 µg of some alkaloids produces a positive inotropic action in the heart-lung preparation (Krayer and Acheson, 1946)

Benforado (1957) has compared the cardiac actions of various tertiary amine ester alkaloids and has concluded that the highest molar potency with regard to positive inotropic action in the heart lung preparation is found in the tri and tetraester alkaloids such as protoveratrine A and B germitetrine B and neogermitrine. He also found that the doses which produce cardiac arrhythmias vary from 2.5 to 39 times the minimal inotropic doses.

Benforado (1958) has recently demonstrated that veratridine directly increases contractile force of isolated ventricle strips of the rat. After the initial increase in systolic tension, resting tension then increases. This is in contrast to the lack of effect of ouabain on resting tension (Section II B 2)

Acetylandromedol resembles protoveratrine in transiently and incompletely reversing the failure of the heart lung preparation (Cotten *et al.*, 1956) Like the veratrum alkaloids, it produces cardiac arrhythmias at doses somewhat above the inotropic doses.

The secondary amine veratrum alkaloids, such as veratramine, have virtually no effect on cardiac contractility In doses as high as 10 mg in the canine heart-lung preparation there is neither positive nor negative inotropic action (Krayer personal communication)

2 *Indirect Effects on Contractility*

In addition to the direct action on the heart, the tertiary amine ester

alkaloids and acetylandromedol indirectly stimulate the heart presumably by a centrally mediated sympathoadrenal discharge. For instance, Cotten and Walton (1951) have shown that the increase in contractile force resulting from the intravenous injection of large doses of veratridine and cevadine in vagotomized dogs is largely prevented by adrenalectomy or by the administration of large doses of the adrenergic blocking drug, dibenamine, in doses which abolish the cardiac stimulation of epinephrine. They concluded that the alkaloids stimulate the heart *in situ* by both direct and indirect actions, the latter resulting from release of catechol amines from the adrenal medulla. Evidence for adrenal medullary stimulation has been described for veratridine (Mendez and Montes, 1943) and for protoveratrine (Krayer *et al.* 1944)

Acetylandromedol also increases the contractile force of the heart through similar indirect actions. Cotten *et al* (1957) found that it augments contractile force in the open-chest dog after the prominent circulatory reflex effects have been prevented by vagotomy and carotid sinus neurotomy The increased force induced by moderate doses in this preparation is predominantly indirect, since the effect is virtually abolished by procaine infiltration of the cervical spinal cord. Inasmuch as neither adrenalectomey alone nor cardiac sympathectomy alone prevented the contractile force response, it was concluded that acetylandromedol produces a sympathoadrenal discharge as a result of stimulation of centers in the brain.

C. Mechanism of the Direct Action on Contractility

Little can be said regarding the mechanism of action of veratrum alkaloids on myocardial contractility because of the paucity of experimental work. They have been compared to the cardiac glycosides on the basis of their direct positive inotropic actions in low doses followed by cardiac arrhythmias at higher doses. Also, Wollenberger (1949) and Reiter (1950) found that the tertiary amine alkaloids increase O_2 consumption of myocardial slices, but not of homogenates, in a manner similar to that of the glycosides. In high concentrations, the alkaloids depress cellular respiration. In contrast to the tertiary amine ester alkaloids, veratramine, a secondary amine alkaloid lacking cardiac inotropic action, produces only depression of O consumption of myocardial slices (Reiter 1950)

Unlike the glycosides, veratrum alkaloids appear to have little effect

on contractile proteins. Straub *et al* (1948) found that veratrine depresses the rate of polymerization of myocardial G-actin to F actin According to Robb *et al* (1955), veratridine causes contraction of skeletal muscle actomyosin fibers in the absence of ATP but the effect was no greater than that caused by non-specific agents such as distilled water histamine, and acetyl β methacholine and was much less than the response to ATP or to calcium.

The staircase phenomenon of the frog ventricle is inhibited by veratrine (Szent-Györgyi, 1953, p 97) and by protoveratrine A and acetylandromedol (Hajdu, personal communication) Hajdu proposes that veratrine and related substances increase the efflux of potassium from the cell with each beat, resulting in reduced intracellular potassium content. This is in contrast to the inhibition of K influx produced by glycosides (Section II C, 4) However the relation of potassium loss to the effect of veratrum alkaloids on contractility is not clear since Witt and Jaeger (1958) found that these two parameters can be dissociated after administration of veratridine to isolated rabbit auricles.

Cotten and Walton (1951) have suggested that the effect of veratrum alkaloids is like that of sympathomimetic amines on cardiac contractility In the intact animal, the indirect action of the alkaloids indeed resembles that of epinephrine, since this action is mediated through a sympathoadrenal discharge. Whether or not the direct effect on myocardium is sympathetic in nature, however, is not clear

Robb and Witt (1958) have presented evidence that veratridine produces a "veratrine response" in the isolated rabbit heart analogous to that seen in skeletal muscle. They found that veratridine prolongs systole, decreases relaxation and causes secondary contractions which are accompanied by repetitive electrical discharges and slower repolarization. This agrees with the suggestion that myocardium is nonsyncytial and that tetanic responses are possible (Robb 1949 1952 Moore and Ruska, 1957)

D Summary

The tertiary amine ester alkaloids of veratrum and of related plants increase myocardial contractility both directly and indirectly The indirect effect on the heart appears to be due to a sympathoadrenal discharge, probably as a result of stimulation of sympathetic centers in the brain. The mechanism of the direct action is only poorly under

of the isolated frog heart (Ringer and Sainsbury 1883 Thomas, 1957) and the cat papillary muscle (Garb 1951) Strontium also increases the force of contraction of both the frog heart and cat papillary muscle in a calcium deficient medium, but strontium differs from calcium by prolonging systole (Thomas, 1957 Garb, 1951) In the frog heart, at least, the prolongation of systole by strontium appears to be due to a loss of intracellular potassium caused by this ion (Thomas, 1957) Addition of calcium restores to normal the strontium-induced prolongation of systole (Ringer and Sainsbury 1883 Garb 1951 Thomas, 1957) indicating a form of antagonism between these two ions. The presence of calcium in the medium prevents the loss of intracellular potassium and prolongation of systole induced by strontium in the frog heart (Thomas, 1957) and presumably calcium restores the prolonged systole to normal by restoring intracellular potassium to normal. The latter assumption is supported by the fact that, in the absence of potassium in the medium, calcium not only fails to reverse the effects of strontium but actually further prolongs systole (Thomas, 1957)

5 *Barium*

Substitution of barium for calcium results in gradual reduction and eventual cessation of the contractility of the frog heart large amounts of barium induce systolic contracture, which is not obviated by addition of potassium (Ringer and Sainsbury 1883) The auricles and ventricle of the turtle are also depressed by barium (Wedd and Blair 1945) Small doses of barium have little or no action on contractile force of the cat papillary muscle, but high concentrations depress contractility (Krop 1944) Intravenous injection of barium chloride in anesthetized dogs increases cardiac contractile force (Walton and Brodie, 1947) but most of this effect can be attributed to sympathoadrenal discharge (Woods *et al.*, 1956)

6 *Magnesium*

Magnesium has little effect on myocardial contractility Cat papillary muscle contracts normally in magnesium-free medium and in medium containing ten times the physiological concentration (Garb, 1951) It has been reported however that increased serum levels of magnesium in the dog heart lung preparation decrease output and competence of the heart, suggesting a depressant action on contractility (Stanbury and Farah 1950)

B Mechanism of Action

No attempt has been made to review comprehensively the effects of ions on enzymatic systems involved in the production of energy for myocardial contraction. This discussion is designed to indicate some major areas in which ions have been shown to influence contractile proteins and the final stages of energy supply for contraction.

1 *Sodium and Potassium*

Both sodium and potassium induce polymerization of actin extracted from cardiac muscle, and this reaction proceeds most rapidly in concentrations isosmotic with blood (Szent-Györgyi 1951) Combined sodium and potassium, in concentrations found in blood have a greater effect on the rate of polymerization than either ion alone, while increasing the concentration of either ion above that found in blood decreased the rate of polymerization. In the absence of potassium the shortening of skeletal muscle myosin B induced by ATP is less than when potassium is present, the increases in the magnitude and rate of shortening are approximately proportional to the increases of potassium concentration from zero to 0 1 *M* (Bowen, 1951)

The influence of changes in intracellular potassium content on the force of contraction of the frog heart is seen in the experiments of Hajdu (1953) who has studied the staircase phenomenon of Bowditch When the potassium concentration of fiber water is 107 meq per liter electrical stimulation evokes no contraction, whereas reduction in potassium concentration by only 3 meq per liter changes isometric contractile force from zero to maximum Reduction in the internal sodium concentration has the same effect. Further reduction in intracellular potassium by 6 to 7% produces systolic contracture, even in the absence of electrical stimuli. Hajdu (1953) found that decreased potassium was associated with concomitant loss of water so that the intracellular concentration of potassium was unchanged, suggesting that the contractile proteins are sensitive to changes in the total amount, rather than the concentration, of the ions in the cell.

With each contraction of the perfused frog or turtle heart, a measurable quantity of potassium is rapidly extruded from the cells and is slowly regained during relaxation (Wilde *et al.*, 1955 Hajdu, 1957) The longer the diastolic interval the greater is the re-entry of potassium into cells. When the frog heart contracts at a slow rate, the force of

contraction is small, but with fast rates and less time for re-entry of potassium during relaxation contractile force increases. Force of contraction under these conditions is inversely proportional to the intracellular potassium content and, as Hajdu (1957) states, the tension developed is a sensitive indicator of intracellular ionic balance.

The staircase phenomenon of the frog heart is abolished by addition of serum obtained from a variety of species (Hajdu and Szent-Györgyi, 1952) and is not seen in intact animals. However Conn and Robertson (1955) who studied the kinetics of potassium transfer in the heart of anesthetized dogs, concluded that, with each beat of the heart, there is probably a rapid outflux and a slow influx of potassium of equal magnitude so that potassium content of the cells remains unchanged.

2 *Calcium*

According to Szent-Györgyi (1951) at least four atoms of calcium are contained in each molecule of actin, but the significance of this is obscure. Calcium and potassium have been reported to have a synergistic effect upon the polymerization of globular actin to form fibrous actin, with maximal activity occurring at the physiological ratio between these two ions (Straub *et al.* 1948) On the other hand, both the rate and magnitude of shortening of skeletal muscle myosin B, in response to ATP are diminished in the presence of calcium (Bowen, 1951) Robb *et al* (1955) found that calcium chloride in a concentration of 1 8 *M* induces shortening of actomyosin threads to a greater extent than that produced by 0.3% ATP However this effect may be nonspecific, secondary to the high concentration of the calcium. Smaller concentrations of calcium chloride, as well as a variety of other substances including acetyl β methacholine, histamine alcohol potassium chloride, and distilled water cause only minimal shortening of actomyosin threads

Calcium increases contractile force of the frog heart both under aerobic and anaerobic conditions, as do cardiac glycosides and epinephrine (Ellis, 1953) (Sections II C 2 and III C, 1) High energy phosphates are required for this effect. In this connection, it is of interest that calcium enhances the ATPase activity of myosin and actomyosin (Engbaek, 1952) Thus, one possible explanation for the increase in contractile force induced by calcium may be an increase in energy available for contraction through more extensive breakdown of ATP

Calcium may also produce its effect on contractility by an action at the cell surface as Niedergerke (1957) has postulated. Based upon studies of the rate of change in contractile tension of the frog heart relative to changes in net movement of calcium between tissues and extracellular fluid, Niedergerke concluded that a certain quantity of calcium is taken up reversibly by a superficial layer of the heart while contractile tension changes However the intermediate steps between such events at the cell surfaces and the increased contractile tension evoked by calcium remain to be defined.

Another possible explanation for the stimulant action of calcium was suggested by Lissak (1938) who showed that Ringer solution containing excess calcium, unbalanced by potassium, causes the release of a sympathinlike substance from the frog heart, an increase in contractile force of a second frog heart was observed upon addition of this material to the medium. Harris and Madjerek (1948) also arrived at a similar conclusion, but questioned the concept since calcium and adrenalin differed in their abilities to evoke ventricular ectopic systoles.

In the intact dog, the positive inotropic effect of sympathomimetic amines and of sympathetic nerve stimulation can be dissociated from that of calcium by utilizing the dichloro analog of isopropylnorepinephrine, described in Section III C, 4 The fact that the effects of adrenergic stimuli are completely prevented while those of calcium are not suggests that the augmenting action of calcium is not due to release of catechol amines, either from sympathetic nerve endings in the heart or from the adrenal medulla.

3 *Magnesium*

The important rôle magnesium plays in enzymatic processes supplying energy for contraction is well known and will not be dealt with here (for references see Engback, 1952) Magnesium also has an important effect on the contractile proteins. Szent-Györgyi (1951) has shown that polymerization of actin does not occur in the absence of magnesium, but, in the presence of this ion the rate of polymerization is proportional to the amount of magnesium added. In addition, the rate and magnitude of shortening of myosin B threads from skeletal muscle of the rabbit are increased in the presence of 10 m*M* of magnesium (Bowen, 1951) The latter result may be related to the augmenting action of this ion on actomyosin ATPase activity since it has been shown that magnesium, in concentrations of between 1 and 5 m*M*

enhances the phosphatase activity of actomyosin (Engbaek, 1952) On the other hand, even very low concentrations of magnesium inhibit ATPase activity of pure myosin, and Mommaerts and Seraidarian (1947) concluded that magnesium probably inhibits ATPase activity of myosin under conditions found in living muscle. The fact that magnesium has little or no effect on the force of contraction of cat papillary muscle, either when absent or present in high concentrations (Garb 1951), suggests that the cell membrane is relatively impermeable to this ion or that skeletal and cardiac muscle are different.

C. Summary

Calcium and strontium increase, while potassium and barium decrease, myocardial contractility In contrast, sodium and magnesium have only minor effects on contractility Antagonistic effects occur among the actions of some of these cations as in the case of calcium and potassium. Cations also have measurable effects on the contractile proteins, energy sources for contraction, and the cell membrane of cardiac muscle, but for the most part, these actions have not been related to the contractile process *in vivo*

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CHAPTER III

Virus Infections

E. J FIELD

I INTRODUCTION

Muscle tissue in general is not very receptive to virus infections, with the outstanding exception of the recently discovered Coxsackie group Levaditi (1926) has pointed out that infections of mesodermal derivatives (les mésodermoses) are produced by and large by bacteria, fungi, spirilla, or protozoa, i.e. by microorganisms which are visible in the light microscope, while those of the ectoderm (les ectodermoses) are due most often to filterable viruses. While muscle itself may not react even to the introduction of virus by direct injection, generalized injection may nevertheless follow Diseases such as herpes and rabies can in fact regularly be produced by intramuscular inoculation of the virus without the occurrence of local changes On the other hand, certain viruses which are essentially "neurotropic" have shown themselves capable of producing lesions both in voluntary and in cardiac

muscle. It has also come to be recognized that epidemic myositis (Bornholm Disease) is viral in origin.

II. SPREAD OF AN INTRAMUSCULAR INJECTION

If herpes or rabies virus containing brain emulsion be injected intramuscularly, general infection results in a wide range of laboratory animals such as mice, rabbits, dogs, monkeys, etc. An inoculum of the order of 0.5–1.0 ml. of 10% brain suspension is commonly employed for larger animals and 0.2 ml. for mice. Experiments with such volumes of india ink or Weed's Prussian Blue solution show that a so-called "intramuscular" injection spreads widely in the tissue spaces between muscle fiber bundles and in the lymphatics of the fascial planes. It is indeed because of this immediate spread into the septal lymphatics that absorption from a therapeutic intramuscular injection can take place rapidly.

III. EFFECTS OF INOCULATION OF NORMAL BRAIN SUSPENSION

Normal sterile tissue suspension (10% is commonly used) when injected into a muscle produces an interstitial inflammatory infiltration with lymphocytes and some polymorphs. Rustigan and Pappenheimer (1949) as a preliminary to their work on the local intramuscular effects of various viruses, investigated the evolution of the "lesion" produced by normal brain tissue, using their standard 0.2 ml. inoculum. They reported an early and often quite intense local reaction with edema and polymorph and mononuclear infiltration. Muscle fibers adjoining the injection site sometimes showed necrosis but restitution followed, so that little sign of the reaction might persist after a few days. They found, too considerable variation in the intensity of the reaction provoked by individual normal brain preparations. This obviously makes more difficult still the assessment of the significance of the relatively mild reactions described after intramuscular inoculation of certain viruses.

IV. HERPES

No significant changes occur in striated muscle when animals are infected by routes other than the intramuscular one, and then only in the muscle which has been directly inoculated. If the masseter muscle of a rabbit so inoculated is examined when encephalitis has become established, the muscle appears normal or perhaps a little

swollen and rather gelatinous. Histologically many muscle fibers have lost their striations and taken on a uniform eosin staining Scattered columns of lymphocytes are present between the fibers but polymorphs are absent. Accumulations of lymphocytes may be found around small nerve bundles, the fibers of which, however remain unharmed. No herpetic inclusion bodies are present and as a whole, the changes are nonspecific and resemble those found when a suspension of normal brain is inoculated in a similar way i.e. there is no specific "take" in the muscle fibers (Field 1952)

V RABIES

Alezais and Bricka (1904a) claimed to find generalized and early lesions in the muscles of infected rabbits in the form of accentuated longitudinal striation, increased numbers of nuclei and, later areas where striation was absent. They described the changes as variable, but most marked in the muscles of the hind limbs (Alezais and Bricka, 1904b) Altogether these changes seem unconvincing and appear to fall within the range of "normal" appearances of striped muscle commented upon in Volume III Chapter V The most thorough work was carried out by Babès (1912) who reported the microscopic lesions to be "peu prononcées," with slight localized proliferations of sarcolemmal nuclei and moderate thickening of the walls of the smallest blood vessels. Field (1951) found that after intramasseteric injection of rage-fixe virus, some muscle fibers presented a hyalinized appearance and stained uniformly pink with eosin, while here and there were islands of muscle fibers containing centrally placed nuclei. There was a moderate infiltration with lymphocytes. It may be concluded that rabies, like herpes, does not produce any characteristic lesion of striated muscle. With street virus, the Negri bodies characteristic of the disease are not seen within muscle fibers.

VI. COXSACKIE GROUP OF VIRUSES

Most interest in lesions of muscle produced by viruses centers around those associated with the Coxsackie group. Their story is now well known (Dalldorf, 1955) but the main facts may be briefly recalled.

A. Historical Note

In 1947 in the presence of a poliomyelitis epidemic, a new virus was isolated from the stools of two affected children in the village of Cox

sackie N Y The distinguishing character of this new virus was an ability to produce a paralyzing disease in suckling mice but not in adult animals. In a preliminary report, Dalldorf and Sickles (1948) found this virus to produce severe and widespread degeneration of skeletal muscle cells, with loss of striations, eosinophilic hyalinization, and fragmentation. Smooth muscle and myocardium was spared. At this stage, no changes were reported in the central nervous system. Curiously enough, it was later found possible to produce severe destructive lesions in the pancreas of adult mice with Coxsackie virus (Conn-5) without changes in the central nervous system, heart, fat, or muscles such as were found in suckling animals (Pappenheimer *et al.*, 1951) Viruses similar to the original Coxsackie specimens have now been isolated in many parts of the United States and in other centers all over the world, and have been called the Coxsackie group of viruses, or C viruses.

B. Groups and Pathogenicity

As was to be expected it was soon found possible to split the original "virus" into many subgroups, chiefly by the labors of Dalldorf and his group on the one hand, and Melnick and his co-workers in the United States on the other (Dalldorf *et al.* 1950 Dalldorf, 1955 Melnick, 1950) It is now recognized that Coxsackie viruses are distinct from those of poliomyelitis, constituting a distinct antigenic group While it is true that other viruses will also produce muscle lesions in suckling mice similar to those produced by the C group (e.g Columbia-SK and MM) they affect adult animals, while the pathogenicity of C viruses is ordinarily limited to newborn animals.

Like poliomyelitis viruses, the members of the Coxsackie group are small viruses (about 20 mμ in diameter) and show similar seasonal and geographical distribution resistance to physical agencies, and general occurrence in the throat washings and feces of infected individuals.

On the basis of the experimental disease producted in suckling mice and hamsters, C viruses are subdivided into two main groups—A and B Each of these has been further subdivided into serological types, so that altogether some score of different strains are known. The outstanding difference between groups A and B is that the former will regularly produce widespread muscular lesions in immature mice or hamsters without damage to the central nervous system, viscera, or fat body while group B viruses are less consistent in their effects. The

muscle lesions which develop are focal in character, the nervous system is commonly involved, as is also the interscapular pad of fat. Sometimes also the pancreas may undergo necrosis. On the whole, group B viruses produce less effect in muscles than do those of Group A, and their most characteristic lesion is produced in another mesodermal derivative—the interscapular pad of fat.

C. Clinical Considerations

From the clinical point of view, viruses which turn out to be of the Coxsackie type when tested on immature mice are found associated with an ever increasing number of different conditions (Ash, 1958) Thus group A viruses have been isolated from cases of brief febrile ill ness occurring in young children during the late summer months (in either the presence or absence of an epidemic of clinical poliomyelitis) and featuring headache, neck stiffness, tenderness of the muscles, together with small blisters which later leave ulcers on the fauces and palate so that the whole pictures has been termed "herpangina." Less arresting are cases of so-called "Summer Grippe" and "Three day Fever " in which myalgia is again a feature. There seem to be many clinical conditions for the most part mild, from which Coxsackie viruses can be isolated, but which yet remain to be clearly grouped from the standpoint of the physician.

Group B viruses have been isolated from outbreaks of epidemic pleurodynia or myalgia (Bornholm Disease) characterized by febrile illness, with headache, sore throat, perhaps some cough or vomiting and a severe stabbing pain in the side of the chest or occasionally in the abdomen or a limb Sometimes signs of an aseptic meningitis are super added (Rhodes and van Rooyen, 1953) Recently cases of myocarditis in new born infants due to Coxsackie group B virus have been reported and a group A virus has been isolated from the feces of cases of Guillain Barré syndrome. The significance of the latter cases has not yet been established Myocarditis has also been reported (on both clinical and pathological grounds) in cases of poliomyelitis (see Section VII)

Finally since Coxsackie viruses may be isolated during an epidemic of paralytic poliomyelitis, there is the possibility that their presence may tip the balance one way or the other with respect to the development of the disease Evidence that this might be so has been found by Dall dorf (1951) and Sacerdote de Lustig and Brieux (1956) and by Dömök (1957)

D. Experimental Infection of Newborn Mice

The outstanding feature of the Coxsackie group of viruses from the pathological point of view is that they will affect the skeletal muscles of immature hamsters and mice. Rowe (1953) working on the assumption that the biochemical transformations in denervated muscle might resemble those of immature muscle (Mirsky and Wertheimer 1942; Schapiro 1943) found that group A virus could proliferate in the gastrocnemius muscle of the adult Swiss white mouse 3 days after denervation, while it failed to do so in the intact muscle. The virus could be carried serially from muscle to muscle which had been denervated.

The more important pathological features of virus myositis will now be considered. Detailed analyses of the lesions produced by group A and B viruses have been given by a number of authors (Armstrong et al., 1950 Melnick and Godman, 1951 Gifford and Dalldorff, 1951 Godman et al., 1952a, Aumonier 1952) and their descriptions have been collated in what follows.

1 *Group A Infections*

In this group, major pathology is found in skeletal muscles, while there is an absence of encephalitic signs (convulsions) Death follows within a day or two after the onset of weakness. The muscle lesions are sufficiently extensive to be seen with the naked eye when the muscle is cut longitudinally as opaque white streaks scattered here and there The lesions are widespread, limbs, diaphragm, scalp, masseter etc., all being affected.

2. *Group B Infections*

Here, encephalitic symptoms are more in the foreground and the mice may present generalized spasms and tremors of the limbs and tail. In contrast with group A, it is in the central nervous system that macroscopic changes in the form of congestion or even liquefaction of the cerebral hemispheres may be met with, while the muscles apparently remain normal. The interscapular pad of fat may however be unusually pale or congested. While in group A infections, almost every muscle of the body is found to be affected when examined microscopically the lesions in group B cases are scattered and focal, and may have to be searched for if not immediately lighted upon. It is possible for

example, that they may have been particularly sparse in the newborn mice described by Pappenheimer *et al* (1950) The microscopic changes produced are, however identical in both groups and may therefore be described together, though individual strains of virus may produce minor differences in histology mainly of degree (Godman *et al.*, 1952)

E. Pathological Lesions

1 *Degeneration*

The first stage is a degeneration of the muscle fibers, pre-eminently segmental i.e. abruptly demarcated regions of obvious change may occur in the course of an otherwise normal muscle fiber The segmental character of the lesions is curiously similar to the Gombault-Stransky type of degeneration in peripheral nerves seen in various pathological conditions, mainly intoxications. No explanation is available for the phenomenon in either case, though it no doubt depends upon subtle biochemical differences which at the moment escape us (Field 1957)

Accentuation of A discs is an early and transient or inconstant feature and is soon accompanied by proliferation of sarcolemmal nuclei The Z discs are said to be at first unaltered, but later they too disappear when myofibrillar thickening takes place. The longitudinally running myofibrillae become accentuated and then obviously thickened passing off on either side into still normal appearing muscle. The sarcoplasm in which the myofibrillae are embedded becomes strongly and uniformly eosinophilic, though at first it may present areas of mottled basophilia. Exactly the same sort of changes may be observed in the neighborhood of a septic focus in muscle and the changes do not seem to be specific. Affected segments may be folded or "fractured" in appearance, perhaps due to an increased rigidity as compared with adjacent unaffected and still contractile fibers. Karyolysis and karyorrhexis take place and the affected segment becomes converted into a waxlike homogeneous mass. Regeneration changes appear very early and this is an outstanding feature of the pathology in these new born mice The course of the degenerative changes in the muscle fibers is also very rapid and many hyalinized segments are to be found on the first day of clinical signs of paralysis.

2. *Inflammation*

This is the natural sequel to degeneration and follows its usual course, with edema, cellular outpouring (histiocytes, polymorphs, and lympho-

cytes) usually reaching its height in about 24 hr., at which stage mononuclear cells predominate. Individual virus strains vary in the degree of inflammation which they evoke.

The mononuclear scavenger cells remove degenerated muscle protoplasm, leaving sarcolemmal tubes which are the directing scaffolding into which regenerating muscular buds can later grow The sarcolemmal tubes are seen to be filled with many nuclei derived from proliferated sarcolemmal cells, histiocytes, and beginning ingrowing muscle sprouts Attempts have been made to distinguish the histiocytes by pretreatment with trypan blue in experimental muscle lesions (Forbus, 1926) and the point is discussed in Volume III, Chapter V The sarcolemmal tubes found in Coxsackie infection are similar to the "Sarcolemmschläuche" described by Waldeyer (1865) in nonvirus destruction and also to the reconstitution of muscle discussed in the next chapter *Phagocytes, from whatever source they are derived,* laden with detritus, emerge from the tubes and a reaction is found in the local lymph nodes. Preparations for regeneration begin very early even before inflammation has had a chance to clear away the necrotic material.

3 *Regeneration*

This takes place to an extraordinary degree, despite the persistence of a high virus titer in the affected muscle (Melnick and Godman, 1951) and originates possibly in part from some of the proliferated and surviving sarcolemmal nuclei within the tubes, but mainly from the adjacent ends of surviving muscle segments. It would seem that regeneration is therefore chiefly of the "continuous" type rather than of the "discontinuous" or "embryonal" type discussed in Chapter V

At the ends of surviving muscle fibers bordering on the necrotic segments, there is a well marked proliferation of nuclei which become elongated and tend to arrange themselves in line, acquiring as they do so a clear and deeply staining mantle of cytoplasm. In this way a syncytial protoplasmic strand is formed which grows into the sarcolemmal tube, now mostly cleared of debris. Often the muscular outgrowth takes place at the periphery of the healthy muscle fiber but sometimes the healthy segment tapers off into a "terminal" syncytial outgrowth The muscle plasmodial slips which grow in often bifurcate. Nuclear division seems to be by amitosis, while that of the sarcolemmal nuclei is mitotic. The sarcolemmal tubes clearly exert a guiding influence on the in-

growing muscle sprouts in much the same way as neurilemmal tubes do upon axis cylinder sprouts during nerve regeneration. There may be much cellularity at the height of the regeneration process, so that the microscopic appearance may come to resemble a myosarcoma. It seems that many of the muscle sprouts fail to find sarcolemmal tubes which can be occupied, and so come to nothing

4 *Differentiation*

Restitution of damaged muscle fibers is for the most part complete about a week after the onset of signs. The muscle sprouts are at first thin and strongly basophilic, with centrally placed nuclei in line. In general, maturation changes follow the course occurring in normal ontogeny Thus longitudinal striae appear first; usually by the second day after the onset of signs they are definite, i.e. even before marked ingrowth into the sarcolemmal tubes has had time to take place. At the same time the sarcoplasm increases in amount and becomes eosinophilic as myohemoglobin begins to accumulate within it. The longitudinal striations increase in number and show accentuations along their course which are the beginnings of A discs. The nuclei which are still centrally placed begin to space out from one another and about the fifth day Z discs and sarcolemmae begin to appear so that by the end of the week the muscle fibers look like well developed young structures. Only their slimness and the central location of their nuclei indicate their newness. Remnants of destroyed muscle tissue may still be scattered about, here and there. The process of striated muscle fiber degeneration and regeneration consequent upon Coxsackie virus infection is essentially the same as that which follows fiber degeneration from other causes, e g direct intramuscular inoculation of toxic chemicals (Forbus, 1926) ischemia (le Gros Clark, 1946) etc. The time scale on which the changes here discussed occur is a rapid one, partly no doubt because they are taking place here in neonatal animals. The temporal sequence of changes in older animals is summarized in Volume III Chapter V

5 *Histochemical Changes*

It has been pointed out above that a striking feature of the muscle changes is the discontinuous nature of the initial lesions, affected segments being found along the course of apparently normal fibers. Some

histochemical observations of the changes brought about in muscle by Coxsackie virus infection have been made in recent years but they are largely unconnected, and a co-ordinated picture must wait upon a fuller development and interpretation of techniques. It has, for example, been recorded that birefringence is increased in the early stages of infection but that no definite A discs can be seen, that basophilia is increased in the hyalinized segments but that this is apparently not associated with either deoxyribo- or ribonucleic acids that phosphate and phosphatase are variable that lipids may appear as tiny granules that with increasing length of infection there is a potassium and creatinine depression (Gifford and Dalldorf, 1949) and sodium elevation (Gädeke and Walenberger 1952) and that glycogen content is diminished (Albrecht 1954) On the other hand, it has been claimed that alkaline and acid phosphatase activity are unaltered (Kausche *et al* 1951) and that also unaltered are both inorganic and total phosphorus levels (Albrecht and Sauthoff, 1954) Ferric iron has been found in affected muscles (Godman *et al* 1952) While these authors and also Pette (1952) have claimed to demonstrate calcium in the lesions, their results have not been substantiated (Sauthoff 1956) More recently, Albrecht and Gädeke (1956) have claimed an increase in inorganic phosphorus in paralized muscle, while the acid soluble phosphorus and phospholipids remain unaltered and suggest that the change is associated with interference with the formation of the phosphates so important as a source of energy in muscular contraction The change is not found in the first 24 hr after infection. The biochemical changes in muscle may be so severe as to lead to kidney lesions in infected suckling mice resembling those seen in "crush kidney" when striated muscle has been extensively damaged (Gädeke, 1952)

It is interesting that some selective interference with the biochemical transactions of heart and skeletal muscle leading to necrotic changes similar to those described above can be brought about in rabbits mice, and rats by intravenous injection of papain, trypsin, ficin, or streptokinase (Kellner and Robertson, 1954a) The activity is apparently due to proteolytic activity of these enzymes in the case of streptokinase plasminogen seems to be converted into the proteolytic enzyme, plasmin They also found that streptococcal proteinase had a similar selective necrotizing effect (Kellner and Robertson 1954b)

F Bornholm Disease
(Epidemic Myalgia or Pleurodynia Devil's Grip)

1 *Historical Note*

The disease takes its name from the epidemic on the island of Bornholm described by a Danish general practitioner whose family developed the condition while on holiday there in 1930 and his monograph on the subject appeared in 1934 (Sylvest, 1930a, b 1934) About the same time, too Pickles described cases in Yorkshire, England (Warin 1956) Actually the disease seems to have been known for much longer than this, having been first described by Finsen (1874) as occurring in two epidemics in Iceland in 1856 and 1863 and since that time outbreaks have occurred in many countries of northern Europe and North America (Finn *et al.*, 1949) Recent outbreaks in this country have been described by Warin *et al* (1953) Disney *et al* (1953) and Swain and Mitchell (1953)

Following the isolation of Coxsackie virus in 1948, Curnen *et al* (1949) reported briefly on the presence of the same agent in the feces of four cases of apparent epidemic myalgia. Since that time the association has become firmly recognized.

2 *Clinical Features*

Clinically Bornholm disease is characterized by the sudden onset of severe stabbing pain usually in the chest or abdomen, less commonly in the trunk or head and neck. A varying degree of moderate pyrexia is usually present for a few days, and headache with neck stiffness and some photophobia may suggest a benign meningitis. Muscle tenderness may be present in the affected region. Generally the condition does not last more than a week or so

3 *Pathology*

The condition is not fatal, but the findings in muscle biopsy have been reported upon by Lépine *et al* (1952) In two adult cases they found the same sort of lesion in muscle fragments removed from the site of maximal affection as occur in newborn Coxsackie infected mice. Thus, in one case there were localized interstitial mononuclear cellular infiltrations with few polymorphs, hyaline degeneration with loss of striation of muscle fibers, followed by necrosis and the clearance of sarcolemmal tubes by phagocytes. Coxsackie virus was demonstrated

by carrying over the infection to suckling mice. A second case showed disappointingly few lesions but this may well have been due to the chances of biopsy. Thus, Welborn (1936) found no pathological changes in a slip of latissimus dorsi muscle removed at biopsy from a case during an epidemic in Cincinnati.

Freudenberg *et al.* (1952) have reported upon the lesions due to Coxsackie virus (proven by transfer to immature mice) in a 7½ months old child with congenital deformity in both hips, apparently consequent upon some intra uterine (blood borne) infection with virus. This virus had produced gross muscular changes reminiscent at operation of the naked eye appearances seen in Coxsackie infected baby mice. There was much scar formation and persistent signs of a low grade infection, the condition thus differing from the self contained process which takes place in the new born mouse. The authors remark upon the prolonged presence of virus, which could indeed still be isolated from the stools at nine months after birth. While there can be no doubt now of the association of Coxsackie virus with Bornholm disease, there are also several other conditions in which it may be found and indeed it may be carried by an apparently normal person.

VII. POLIOMYELITIS

So long as poliomyelitis viruses were regarded as exclusively neurotropic, little attention was devoted to possible early changes in muscles which might be of importance for an understanding of the disease process. In more recent years, with the development of a less rigid outlook, muscular changes have been sought for and recorded.

Chor (1933), Horányi Hechst (1935), Sanz Ibanez (1945) and especially Carey and his co-workers (1944; Carey, 1943, 1944) claim to have demonstrated early changes in muscle motor end plates of humans and animals infected with poliomyelitis. In the monkey, Carey (1943) found some end plates to be retracted into ball like masses as early as the first day of paralysis and to stain deeply with gold chloride. He found many to be small and dense, while others were large and granular. He estimated that some 20% of end plates were absent on the first day, but this must be accepted with reserve because of the well known capriciousness of the gold chloride method, in which many of the factors determining the success of impregnation are as yet undetermined. However, within 2 to 4 days, Carey reported 50% of the end plates to have disappeared and that this was followed by centripetal changes in the

motor nerve fibers. This sequence is different from that which occurs when a nerve is sectioned for then changes proceed distally Carey also found the muscle fibers themselves to show lesions beginning in the vicinity of the sole plates and spreading into the adjacent sarcoplasm. Transient "inclusion masses" were found in the neighborhood of the end plates, but only in the early stages of the infection. It must be admitted, however that the assessment of such masses in metallic impregnated material is difficult. There were also lymphocytic infiltrations in the muscles. Other workers have not found such alterations but have drawn attention to the occurrence of sporadic sarcosporidiosis (Hurst, 1929) While there can be no doubt that the main attack of the poliomyelitis virus is on the anterior horn cell (Bodian, 1948, 1949) it is possible that disturbance here may manifest itself very rapidly (with in a matter of a few hours, or even minutes) at the highly labile synapse or motor end plate before changes along the course of the intervening nerve fiber appear

Hassin (1943) described histological changes in the intercostal muscles diaphragm, pectorales major and minor of a boy of twelve who succumbed to poliomyelitis. Varying degrees of homogenization, waxy degeneration, loss of striation, and disruption of muscle fibers were found together with some infiltration of the myocardium but the changes do not seem very convincing Nevertheless, there is some evidence that muscle may be affected early and apparently directly in poliomyelitis infections. Caughey and Malcolm (1950) for instance, found that electromyographic studies failed to reveal motor potentials in muscles which were in spasm, curare failed to relieve spasm, which could, moreover be seen in muscles devoid of voluntary power They therefore suggested that it originated, to some extent at least, within the muscle itself Guyton and Reeder (1950) performed anterior rhizotomy on dogs and observed the development of muscle tenderness to pressure and stretch such as occurs in poliomyelitis suggesting that these features might arise from local pathological changes in the muscle, possibly following the accumulation of metabolic products. Pollock *et al* (1949) maintained there was no true spasm during the development of paralysis but that tenderness and stretch pain occurred more particularly in paralyzed muscles. Successful attempts to isolate poliomyelitis virus from muscle have been relatively few in number Jungeblut and Steevens (1950) succeeded in one of thirteen cases in isolating the virus from puncture biopsy specimens of muscle. It seems

that there is considerable variation in the ability of different human polio strains to produce lesions when injected directly into muscle. Thus, Rustigan and Pappenheimer (1949) found that Columbia SK virus (of Jungeblut) was highly virulent and produced lesions of great intensity. With a standard dose of 0.2 ml of 10% brain suspension, definite lesions were recognized within 15 min. Edema was a prominent feature and was followed in the next few hours by polymorph infiltration and necrosis of muscle fibers, especially those up against the intramuscular septae, whither as has been seen above (Section II, page 86) most of an intramuscular injection finds its way. Lansing virus produced only a slight and transitory effect. True human poliomyelitis virus is in general much less pathogenic for muscle even when inoculated directly (Verlinde, 1952) and produces an interstitial rather than a parenchymatous reaction. Clearly these changes are different from those produced in young mice by Coxsackie virus.

On the other hand, Theiler's GD VII and FA viruses are very pathogenic when inoculated directly into the muscles of mice up to 3 weeks of age. Gross swelling and edema is induced and later the affected muscles appear opaque and streaked with yellow. Histologically there is an intense necrotizing myositis with much the same character as that described above for Coxsackie infections. Destruction of muscle fibers takes place without apparent damage to nerve endings and small nerve bundles. Eosinophilic inclusion bodies with margination of the nuclear chromatin (as found typically in herpes) were seen by Rustigan and Pappenheimer (1949). Apart from these the lesions in general were remarked upon as resembling those found in vitamin E deficiency and ran parallel with marked local proliferation of virus in the injected muscles. Denervation does not interfere materially with virus multiplication or lesion production.

VIII. OTHER VIRUS INFECTIONS OF MUSCLE

Lymphocytic choriomeningitis virus may be the cause of aseptic meningitis in humans and is not uncommonly demonstrable *in feces* of apparently healthy mice. When injected intramuscularly the virus produces an intense edema with a certain interstitial infiltration with mononuclear cells and occasional polymorphs. The lesions are different from those produced by the other viruses investigated by Rustigan and Pappenheimer.

Vesicular stomatitis virus is capable of producing a myositis on local

injection into a limb associated with interstitial infiltration degeneration, and phagocytosis of muscle fibers and proliferation of sarcolemmal nuclei. No characteristic inclusion bodies have been reported (Sabin and Olitsky 1938) Recently Platt (1956) has found that mice up to 5 weeks of age can be infected intraperitoneally with the virus of foot and mouth disease. Skeletal muscular lesions very like those of Coxsackie infection result. Lesions were also found in the heart muscle of these animals (Section IX, E)

St. Louis encephalitis virus likewise can produce such changes in young mice (Peck and Sabin 1947)

Recently an infectious pancreatic necrosis of trout has been described (Snieszko *et al.*, 1957) as accompanied by a focal myositis resembling that produced by Coxsackie Conn-5 virus. In marked contrast, however to what is found in mammals, there is practically no inflammatory or regenerative change. The heart and central nervous system are not affected. No viral studies have yet been published but the authors suggest the condition may be caused by an agent belonging to the Coxsackie group

IX. MYOCARDITIS

A. Idiopathic Myocarditis (Fiedler's myocarditis)

An interstitial myocarditis of obscure etiology has long been known to occur in common experimental animals and in man (Editorial 1951) Fiedler (1900) first described isolated myocarditis in man and the condition has been variously termed idiopathic, interstitial, isolated primary etc and many cases have now been recorded Indeed, they would seem to be increasing in frequency (Saphir and Cohen 1957) but unfortunately virus studies are lacking (Gajdusek, 1955)

Miller (1924) during the course of experiments designed to produce rheumatic changes in the hearts of rabbits and guinea-pigs, found lesions in 60% of "normal" rabbits, in the form of interstitial infiltrations with lymphocytes and endothelial leucocytes, with occasional polymorphs, plasma cells, and eosinophiles. Muscle fibers themselves were not infected nor was any form of inclusion body present. The importance of such findings for the interpretation of experimental results needs no emphasizing and the obtaining of "normal" animals for experimental work has now been recognized as a major problem. Lenke and Loewe (1941) found up to 30% of white Swiss mice to show "spon-

virus in the myocardium of the experimental animal, is its virulence encountered from time to time in the heart muscle of the human new born. "Epidemic myocarditis of infancy" has now become recognized and in some localities seems to have been endemic for years (Stoeber 1952) It is characterized by an acute onset with sudden loss of appetite, vomiting cyanosis, and tachycardia, usually in children about 1 year old. Cardiac dilatation is common at autopsy and polymorph infiltrations (occasionally also lymphocytes) with actual muscle necrosis in the more severely affected foci, have been described. An outbreak occurred in 1953 in Australia in a nursery for newborn infants, with much the same character as that described by Stoeber (French, 1953). During 1952 in the presence of an epidemic of Bornholm disease in Johannesburg a number of babies were striken with myocarditis while in, or soon after discharge from, a local maternity home. The babies were aged between 5 and 17 days. This outbreak of myocarditis has been recorded in full by Javett *et al.* (1956) Other South African cases have been described by Montgomery *et al.* (1955) while in Holland, van Crevald and de Jager (1956) and Verlinde *et al.* (1956) have described a similar epidemic. From both the Dutch and the South African epidemics, group B Coxsackie virus was isolated.

Kibrick and Benirschke (1956) have recorded a case of intra-uterine infection with B virus, type 3 leading to the development of myocarditis associated with meningoencephalitis, the condition proving fatal within 7 days after birth. This case should be compared with the case of Freudenberg *et al.* (1952) which illustrates intra uterine Bornholm disease mentioned above. Coxsackie viruses can on occasion produce a viremia (Dalldorf, 1955) and the above cases demonstrate its ability to cross the placental barrier in muscle in the same way as has been found in variola (Horning 1932) measles (Babbott and Gordon, 1954) poliomyelitis (Bates, 1955) and western equine encephalitis (Shinefield and Townsend 1953)

In Coxsackie infection, the heart of the newborn shows macroscopically only a flabby dilatation. Microscopically there may be patchy edema and infiltration with lymphocytes and a few polymorphs, especially in the inner part of the myocardium. Here and there, patches may be found where muscle fibers have lost their striations and eventually necrosis may have taken place. It would seem that patchy cellular infiltration may precede muscle fiber degeneration—a reversal of the sequence found in Coxsackie invasion of skeletal muscle According to

Javett *et al* (1956) disappearance of the homogenized sarcoplasm leaves empty sarcolemmal tubes, but the existence of the sarcolemma in heart muscle is disputed. These authors also remark on the absence of regenerative changes in their specimens. It is interesting that the absence of regeneration in heart muscle after experimental ischemic lesions has been attributed by Ring (1950) to the absence of a sarcolemmal framework.

The undoubted association of Coxsackie virus with cases of myocarditis in the newborn raises again the possibility of a virus etiology for Fiedler's myocarditis and calls for many more virus studies of the condition. Mogabgab (1957) has recently reviewed the subject of viral myocarditis and has pointed out that the importance to the heart of these infections cannot be assessed without further knowledge. It seems that good functional recovery is made if the attack does not prove fatal at once, but on the other hand repeated scarring from recurrent attacks might well be of ultimate importance in producing a fibrotic myocardium. There is some evidence, too that a direct toxic action on the myocardium may be exerted in some virus conditions, independent of the actual multiplication of virus in the myocardium.

E. Miscellaneous Other Viruses

A number of other viruses of theoretical interest have been found able to produce myocarditis for example, Virus III in rabbits (Pearce 1950) but of more practical importance are the members of the "encephalomyocarditis group" usually termed EMC viruses.

Four viruses are now grouped under this head. They are Columbia SK virus, MM virus, the original Helwig and Schmidt encephalomyocarditis virus and Mengo virus.

Of these Columbia-SK and MM viruses stand very close to one another Col-SK was originally isolated by Jungeblut and Sanders (1940) from the feces of an abortive human case of poliomyelitis and they succeeded in establishing it in white mice through the intermediary of the cotton rat. It has been seen (Section VII) that this virus does not attack skeletal muscle unless direct inoculation is made into the muscle when the pathogenicity is high. But if Col SK virus is passaged 10 times through mice by intraperitoneal injection, then the mice begin to develop well marked myositis and myocarditis by this route (Jungeblut and Steenberg 1950) Interesting species variations in susceptibility also occur for while rhesus monkeys are ordinarily resistant to infec

tion cynomolgus and *Cercopithecus* are susceptible and show well marked myocardial lesions (Jungeblut, 1950) The MM strain is closely related to Col-SK and has very similar properties.

The virus of Helwig and Schmidt has been briefly described in Section IX, B (page 100) and its production of a necrotizing myocarditis in mice and hamsters was confirmed by Warren and Smadel (1946)

Mengo virus was isolated in Uganda from an untreated rhesus monkey which became paralyzed at the Yellow Fever Research Station at Entebbe.

Mengo and the EMC virus of Schmidt are known to be pathogenic for man, and while Col SK and MM are of doubtful significance in human infections, all four are in general infectious for rats, guinea-pigs, hamsters, and mice (Rhodes and van Rooyen, 1953)

There are reports of fatal encephalitis associated with myocarditis in young children, in which the authors have suggested that the EMC virus may be involved (Brenning 1951 Chiari, 1952) though virus studies were not actually undertaken. On the other hand, the virus seems to be definitely associated with at least some cases of "Three day fever" (Smadel and Warren, 1947) Mengo virus has likewise been shown capable of producing a form of encephalomyelitis in man (Rhodes and van Rooyen 1953) and myocarditis has been produced with it in hamsters and mice.

Platt (1956) has recently shown that young mice (up to the age of 5 weeks, after which resistance suddenly develops) show lesions of myocarditis as well as changes in the skeletal muscle when injected intraperitoneally with the virus of foot and mouth disease (Section VIII) The myocardial changes may be large enough to show up as pale areas in the ventricular muscle. Microscopically, there is loss of striation followed by necrosis occurring in patches and accompanied by moderate polymorph and macrophage infiltration As in the case of other viral infections of the myocardium, no evidences of regeneration were seen.

F Summary

It seems likely that some at least of the cases of Fiedler's myocarditis which in the past have been regarded as "spontaneous" may in fact have been due to a virus of the type isolated by Helwig and Schmidt in 1945 It is possible that recurrent attacks of myocarditis might be

responsible for some cases of myocardial fibrosis not otherwise explicable

In the new born infant, Coxsackie B infection may produce a fatal myocarditis. Such cases should be especially looked for during the prevalence of an outbreak of Bornholm disease.

Transient nonfatal myocarditis seems to be by no means an uncommon accompaniment of poliomyelitis, perhaps as a toxic phenomenon rather than one due to actual multiplication of the virus in the heart muscle. Occasionally a severe myocarditis may occur and may be the immediate cause of a fatal circulatory collapse

Intra-uterine infection by transplacental passage of Coxsackie virus may take place as with a number of other viruses and may result in myocarditis (or congenital Bornholm disease which can in turn lead to deformity due to muscle fibrosis)

Many more virus studies of obscure cases of myocarditis are called for before the full significance of viruses as an etiological agent in the condition can be assessed.

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CHAPTER IV

Parasitic Infections

P. C. C. GARNHAM

I. INTRODUCTION

Muscle is subject to infection with two types of animal parasites, protozoa and helminths. Both these infections are widespread in the animal kingdom, but they have nearly always been studied from the angle of the parasite living in muscle rather than that of the muscle infected with a parasite. The dramatic changes undergone by the parasite are undoubtedly responsible for this focusing of attention on one side of the picture, and it has led to a neglect of the changes occurring in the host: even gross physiological disturbances have only been studied in a few instances, let alone the minute histological effects. In hardly any examples is the exact site of development of the parasite known for certain, though it is probable that the muscle fiber is only exceptionally the actual host cell.

As a general rule, the parasite lives in muscle tissue without causing much local reaction. It often becomes encysted and only on rupture of the cyst does any phagocytic response occur. The reaction is normally brief and localized but if the infection persists for a long time with periodic rupture of a cyst and invasion of new muscle fibers, a widespread inflammatory change eventually takes place in the tissue.

thus chronic myocarditis is the final result of a long standing case of Chagas disease.

All types of muscle may be the seat of parasitic infection and it is not unusual for an organism to attack equally skeletal muscle, the heart and the unstriated muscles of the alimentary tract. In a few cases, the parasite has a predilection for special tissues like the diaphragm heart, or tongue. Many infections are asymptomatic, and in the case of man, they may often pass undetected because muscle is not usually included in the histological examination of organs and tissues taken at autopsy It is curious that the muscle of domestic animals, on the other hand, receives more attention, namely in the form of meat for human consumption. Here the flesh is subjected to a visual examination by meat inspectors as a routine, and parasite infection of meat is one narrow aspect of the subject which has been recorded in great detail

The parasites of muscle are by no means confined to human parasites or to parasites occurring in meat destined for human consumption Their range extends to all classes of vertebrates and to many invertebrates some of the most interesting examples are to be found for instance, in insects. All of them exhibit the most important feature of parasite life, namely the attempt of the parasite to live as harmoniously as possible with the host, it is only in exceptional circumstances, or at a late stage of evolution, before a state of natural tolerance has been achieved that gross destruction of the tissue of the host takes place

Muscle provides a unique environment for the parasite, in one respect apparently most unsuitable in another very favorable The repeated contraction and relaxation of muscle subject the parasite to intense stress and strain. It is difficult to understand how the rapidly growing filarial larva for instance, is able to pursue its development in the thoracic muscles of a mosquito in flight—one would expect that nuclear division at least, would be adversely affected Mere muscular contraction must expose the parasite to much buffeting while the periodic interruption of the flow of blood in the vessels supplying the tissue results in equal periods of anoxemia. On the other hand, muscle is the site of rapid metabolism. Here carbohydrate metabolism is at a maximum and nucleic acids and other metabolites are pro l in quantity The parasite has a le opportunity of finding t wants those muscles whi gh metabolic rate are heavily parasitized than in trichinosis and infection is intense in the d tercostals and hea

nothing is known of these facts and the subject is open to an interesting study by a parasitologist who is also a comparative physiologist.

It is possible to describe the effect of parasitic infections on muscle in three ways (1) in relation to the type of muscle (striated, unstriated and cardiac) (2) in relation to the different animals, and (3) in relation to the parasite.

The parasitological approach (3) is the most convenient and the following two sections are devoted to the protozoa and helminths, respectively The more important, i e. the more frequent infections are described in some detail while a few examples only are mentioned concerning the myxosporidia and the extensive group of nematode worms.

II. PROTOZOAL INFECTIONS

Protozoal infections of muscle are either uncommon or are confined to single geographical areas or to special groups of animals. There is one exception viz. *Sarcocystis* a parasite widespread in the animal kingdom and one with which we (as meat-eaters) are in contact nearly every day of our lives. Meat infected with protozoa is rarely considered unfit for human consumption but certain protozoal infections of domestic animals, like *Theileria parva* causing East Coast Fever in African cattle, give rise to gross deterioration in the quality of the flesh, and the carcass may have to be condemned The parasite in such cases does not infect the muscle itself, and infections which have only an indirect effect on the muscle are excluded from consideration here.

Each parasite is described from the point of view of its incidence, its character and mode of entry into the muscle, and the changes it produces in the tissues

A. *Sarcocystis*

Of all parasitic infections of muscle *Sarcocystis* must be the most common the parasite affects many mammals some birds, lizards, and fish. At least 50 species have been described including one from man. The organism is found in skeletal muscle heart muscle and in involuntary muscle in which tissues the organism appears in a cystic form. The cysts are often macroscopic and were easily seen by the early workers who described the parasite in detail over a hundred years ago. In spite of its frequency and the long period during which it has been studied, our knowledge of the life cycle of *Sarcocystis* remains defective. The

thus chronic myocarditis is the final result of a long-standing case of Chagas disease.

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Muscle provides a unique environment for the parasite; in one respect apparently most unsuitable, in another very favorable. The repeated contraction and relaxation of muscle subject the parasite to intense stress and strain. It is difficult to understand how the rapidly growing filarial larva, for instance, is able to pursue its development in the thoracic muscles of a mosquito in flight—one would expect that nuclear division, at least, would be adversely affected. Mere muscular contraction must expose the parasite to much buffeting, while the periodic interruption of the flow of blood in the vessels supplying the tissue results in equal periods of anoxemia. On the other hand, muscle is the site of rapid metabolism. Here carbohydrate metabolism is at a maximum and nucleic acids and other metabolites are produced in quantity. The parasite has ample opportunity of finding the food it wants; those muscles which have a high metabolic rate are even more heavily parasitized than others, e.g., in trichinosis and cysticercosis, infection is intense in the diaphragm, intercostals and heart. Little or

nothing is known of these facts and the subject is open to an interesting study by a parasitologist who is also a comparative physiologist.

It is possible to describe the effect of parasitic infections on muscle in three ways (1) in relation to the type of muscle (striated, unstriated and cardiac) (2) in relation to the different animals, and (3) in relation to the parasite.

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classification of the organism is tentative and even the mode of entry into muscle is unknown.

The parasite grows as a cylindrical or fusiform object, white in color and up to 2 in. in length. Its popular name is "Miescher's tube" and the spores inside the tube are known as "Rainey's corpuscles." Another name commonly used in veterinary medicine is "balbiania," for the cysts found in the esophagus of goats and sheep.

According to Scott (1943) the development of the parasite inside a muscle cell takes place in the following way. An amoeboid parasite enters the cell and the nucleus undergoes repeated division, giving rise to a large number of sporoblasts. The sporoblasts themselves give rise to ellipsoid and later banana shaped spores. The parasite itself has meanwhile grown enormously in size and has become surrounded by a cyst wall which is derived primarily from the muscle and connective tissue of the host. The mature spores measure between 10–15 μ in length, contain a nucleus, a vacuole, and a few granules. They exhibit a peculiar type of movement. They are sometimes called sporozoites or schizozoites. The sarcocyst may become calcified or it may rupture into the surrounding tissue. It is thought that the spores are carried by the blood stream to other parts of the body where they may invade muscular tissue. On the other hand spores have been found in nasal secretion and in the feces and it is possible that the method of transmission from animal to animal takes place via the feces rather than through the consumption of infected meat. The sarcocyst is usually incompletely divided into a number of compartments by septae passing inwards from the cyst wall. *Sarcocystis* has given rise to difficulties of various sorts in diagnosis. Sometimes, when blood is taken from a cow for diagnosing East Coast Fever a sarcocyst may be accidentally penetrated by the needle and the blood become contaminated with the spores. In these circumstances, cattle malaria has been erroneously described with crescent-shaped gametocytes, resembling those of *Plasmodium falciparum* in man. Again, during the search for the exoerythrocytic forms of malaria, curious structures were found in the psoas muscles of infected monkeys, which were at first thought to be the long-sought tissue stage of malaria; a closer examination revealed that the parasites were in fact *Sarcocystis*. Other rarer protozoal parasites of muscle may be confused with this organism such as *Toxoplasma* and *Besnoitia*.

The size and general morphology of sarcocysts varies from species to species, but it is doubtful if all of the so-called species which have been

described are really valid. Certainly when a sarcocyst is transferred from one animal to another a great change in morphology may take place.

The incidence of *Sarcocystis* may be extremely high. Sheep may be 100% infected Lambs show a low rate but sheep of over a year in age may be practically all infected (Awad, 1954) In cattle, swine, and horses, the incidence of the parasite likewise increases with age. The youngest age at which the infection has been detected is 6 weeks, in calves and in lambs. The organism in sheep is known as *S tenella* and it is usually found in the form of nodules in the muscles of the esophagus (Fig 1) In cattle, the species is called *S blanchardi* In cattle, it affects particularly the musculature of the esophagus, tongue, larynx, diaphragm, and skeletal muscles. The camel is commonly infected in India and in Egypt, the parasite being present chiefly in the esophagus and in the heart. The incidence in birds varies greatly Chickens have been found to be infected with this parasite in up to 50% of tissues examined. The most affected muscles are those in the head the neck, and the pelvic region. In the duck, the infection may be quite extensive. Sarcosporidiosis can be pathogenic to mice because of the intensity of the infection nearly all the muscles become parasitized and the animals die. The infection, however both in laboratory and in wild rodents is comparatively uncommon. It is occasionally encountered by accident in the course of post mortem examination of white mice, or less often of rabbits. Ball (1944) found a heavy infection of *Sarcocystis* in lizards in the mountains of southern California. The parasites were present in the muscles of the trunk region Other records from reptiles are confined to the Mediterranean basin.

Sarcocystis has been reported about 18 times in human beings. The sites of infection included the heart, larynx, tongue, and various skeletal muscles (Fig 2) In most of these instances, the parasite was found accidentally as in McKinnon and Abbott's (1955) case from the Sudan where the leg of the patient was removed on account of mycetoma. When sections of the diseased tissue were prepared, the neighboring muscle was found to contain large numbers of sarcocysts. It is possible, that if human muscle were especially examined for the presence of this organism, many more infections might be revealed on the other hand, if the mode of infection of *Sarcocystis* is via the feces, man is unlikely to contract this infection unlike herbivorous animals which graze on land heavily contaminated with their own excreta.

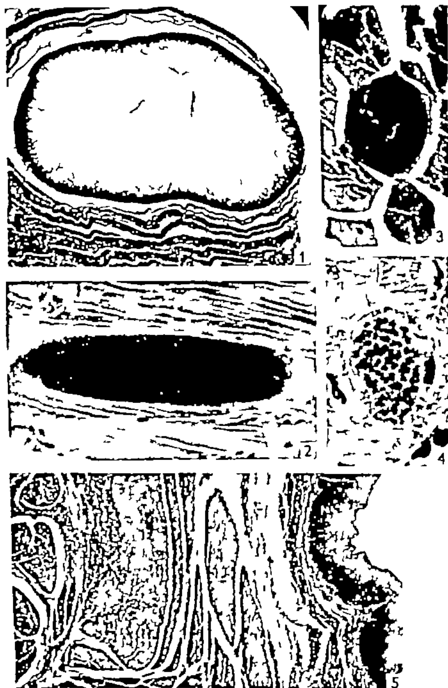


PLATE I

The pathogenicity of *Sarcocystis* as a general rule, is low. Its presence may cause symptoms or even death if a vital organ, for instance the heart, is attacked, and apparently it may occasionally cause an allergic response. McGill and Goodbody (1957) described a fatal case in man associated with widespread periarteritis nodosa, which they suggest is an allergic reaction following rupture of the cysts. When the sarcocysts are present in very large numbers, the intensity of the infection affects the vital processes and may cause death, as in the case of *S. muris* in mice.

During the course of the development of the parasite in the muscle cell, there is usually no tissue response beyond compression of the neighboring fibers but Destombes (1957) has described a severe inflammatory reaction in pigs in the muscle around the *Sarcocystis*. In this animal, the mature cyst does not grow to a greater size than a millimeter: an infiltration of adventitious cells, including numerous eosinophiles, is seen around the parasite, together with much nuclear debris, while beyond this zone there is an inflammatory reaction which extends far into the neighboring muscle fibers. The outer cells are chiefly polymorphonuclear leucocytes, basophiles, and again many eosinophiles. Animals in which this condition has persisted for a long time show a fibrocalcareous myositis ("Myositis Sarcosporidica").

Pugh (1950) described briefly the behavior of an infection in himself in which the peripheral muscles were heavily infected with *Sarcocystis* (Fig. 3). Fleeting swellings occurred over the infected parts and eosinophilia was found in the blood; tissue biopsies revealed a zone of reaction around the parasites.

It is strange that the presence of *Sarcocystis*, even in quite large numbers, can be tolerated by the host, although a highly toxic substance, sarcocystin, exists in the cysts. Paralysis of muscles in infected

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Fig. 1. *Sarcocystis tenella* in esophagus of sheep. Magnification × 28.

Fig. 2. *Sarcocystis lindemanni* in human skeletal muscle (MacKinnon and Abbott's case). Longitudinal section, magnification × 350.

Fig. 3. *Sarcocystis lindemanni* in human skeletal muscle (Pugh's case). Transverse section, magnification × 350.

Fig. 4. Pseudocyst of *Trypanosoma cruzi* in heart muscle of experimentally infected mouse. Magnification × 1000.

Fig. 5. Inflammatory changes in musculature of human esophagus in late stage of Chagas' disease. (Courtesy of Professor F. Köberle.) Magnification × 30.

pigs has been attributed, however to the escape of this substance from the cyst into the surrounding tissue and it is probable that the pathological picture seen in an infected muscle is due to the action of the toxin (sarcocystin) liberated by the parasite.

The muscles of birds are sometimes excessively heavily parasitized with *Sarcocystis* and yet no interference of flight apparently takes place. In ducks, however *S. rileyi* may cause a severe disease resulting in death of the animal (Erickson, 1940) Awad (1954) noted that the muscle of sheep and other domestic animals may be heavily infected, leading to a general loss of condition with emaciation and gross deterioration in the quality of the flesh. In goats the muscles of the larynx may become so heavily infiltrated with sarcocysts that respiration becomes difficult and eventually impossible In an artificial infection of a lamb with *S. tenella*, Awad noted that the heart became involved and that the animal died suddenly from heart failure. *S. tenella* like the other species, causes no cellular reaction in the early stages of infection of the muscle. In old infections when the cysts have ruptured a marked round cell infiltration occurs in the infected tissue while caseation and calcification is the final fate of the parasite itself.

Meat is not normally condemned for human consumption unless the infection is highly generalized and the flesh is watery and heavily infiltrated

In view of the prevalence of this parasite in the animal kingdom, it is desirable that its presence should be excluded when making any researches involving the use or behavior of muscular tissue.

B *Trypanosoma cruzi*

Trypanosoma cruzi is the most pathogenic protozoal parasite of human muscle and the infection is found over extensive areas of the New World. It occurs in all South American countries from the Argentine up to Central America, Mexico and in its insect host and animal reservoirs in the United States of America. *Trypanosoma cruzi* causes the disease known as Chagas disease, or South American trypanosomiasis. In some parts of Brazil the disease is so prevalent that 60% of the population may be infected. It is primarily a disease of wild animals, such as the armadillo in Brazil the bat in Panama, opossums in various parts of South America, and the cavy or wild guinea pig in Bolivia. In all these animals and in small laboratory animals artificially inoculated (particularly baby mice puppies, and kittens) the muscles

are infected in much the same way as in man. The involvement of any particular tissue, however varies from place to place or from strain to strain in the same way as it does in human infections.

The trypanosome is introduced into the skin, eye, or mouth of man during the bite of an infected reduviid bug The bug discharges meta cyclic forms of the parasite in its excreta, and these infect the person. After a local development at the site of entry the trypanosome enters the bloodstream and is carried to various organs and tissues of the body It settles down particularly in the muscle cells of the heart, though frequently various skeletal muscles and other muscular structures are involved

In a heart muscle cell (Fig 4) the trypanosome loses its flagellum and becomes rounded up into a leishmanial form. The parasite then multiplies by binary fission and pushes the nucleus of the host cell to one side. The muscle fiber enlarges, and a pseudocyst is produced which contains many parasites. Such bodies were first seen by Chagas he thought they were the result of schizogony and he therefore called the organism *Schizotrypanum cruzi*, almost certainly on a wrong interpreta tion of events. Flagella grow out of the leishmanial bodies and the organism assumes the crithidial form this in turn becomes a trypano-some by the backward migration of the kinetoplast. The pseudocyst now ruptures and the trypanosomes are set free into the neighboring tissue where they invade new muscle cells or the blood

Up to this point, there has been no local reaction to the presence of the organism except for the enlargement of the invaded cells. Once the pseudocyst has ruptured, however the area rapidly becomes invaded by adventitious cells, consisting chiefly of lymphocytes and histiocytes. After repeated multiplication, a chronic inflammatory state of the heart muscle develops this is followed by fatty and waxy degeneration, which is seen as a mottling of the muscle near the endocardium. Finally a thinning of the ventricle takes place, particularly at the apex of the heart where an aneurism may form.

This chronic condition of the heart, as a rule, does not develop fully for many years after infection usually the individual is infected in childhood and the disease pursues an insidious course until between the ages of 40 and 50 years the infected people start dropping down dead with acute heart failure In certain areas in Brazil, 8% of the population may show gross electrocardiographic abnormalities, while heart block and Adam-Stokes syndrome commonly occur

Other tissues besides the heart may become infected in a similar way Pseudocysts are occasionally found in the skeletal muscles. However it is in the alimentary tract that another type of lesion of considerable medical importance occurs. The muscle of the esophagus, stomach, duodenum and colon becomes infected by the organism pseudocysts develop and on rupture give rise to interstitial inflammation. The adventitious cells spread to the local nervous plexus and extend far along the course of these nerves, destroying them and thus grossly interfering with the normal peristaltic movements of the organ. The organ fails to empty food collects above the lesion and gross muscular hypertrophy takes place. The pathology of this condition has been described in great detail by Köberle (1956) Köberle and Nador (1956) in a series of papers. In their opinion, the heavy damage to Auerbach's plexus with necrosis of ganglion cells, is the effect of a toxin set free on rupture of the pseudocyst. The commonest part of the alimentary tract to be affected in this way is the esophagus (Fig 5) which becomes enormously dilated to give rise to the malady known as "mal d engasgo" in which the patient vomits nearly all food and becomes emaciated. The colon is also commonly affected and becomes enormously hypertrophied it is not unusual for a volvulus to ensue, often with fatal results The stomach and duodenum are less often affected sometimes the muscular wall of the ureter may be similarly infected and give rise to obstruction of the passage of urine. These conditions are known as megaesophagus megacolon, megaureter etc

In the late form of Chagas disease, it is usually impossible to demonstrate the organism in the affected tissue The focal accumulations of adventitious cells present, however a characteristic picture.

The pseudocysts of *T cruzi* measure up to 60 μ in length on superficial examination they could be mistaken for sarcocysts or pseudocysts of *Toxoplasma* but their contents reveal the true nature of the parasite. The condition is unlikely to be met with in laboratory animals, though accidental cross-infections have been known to take place in laboratories in Europe and North America where the trypanosome has been maintained In endemic areas, of course, any suspicious changes involving muscular organs should be investigated to see if they could have a Chagasian etiology

C *Leishmania donovani*

Nests enclosing leishmanial bodies are frequently found in muscle in

Trypanosoma cruzi infections It might be thought, therefore, that the disease leishmaniasis itself would be accompanied by similar lesions but invasion of the actual muscle fiber by this organism is unknown In rare instances the skeletal muscles may be infected with *L. donovani* but as Adler (1940) pointed out, the parasite lies in the connective tissue cells lying between the fibers of voluntary muscle in such circumstances, the muscle may show focal accumulations of lymphoid macrophage cells. Recently Manson Bahr (1957) has reported the presence of *L. donovani* in large numbers in the hearts from acute human cases occurring in an endemic area in East Africa. Undoubtedly in this instance also the parasite grows in lymphoid macrophage cells in the muscular tissue, proliferating in this site, but with no infection of the muscle fiber itself

L. enriettii causes severe cutaneous lesions of the nose of guinea pigs the parasites, which are double the size of *L. donovani*, infiltrate into the deeper tissues to invade the muscles below

D *Besnoitia besnoiti*

A parasite of doubtful affinities is found commonly in cattle of the Transvaal and Rhodesia, primarily in the subcutaneous tissue but often in such numbers that the cysts extend into musculature. For this reason and because of some similarity in morphology the organism was originally placed in the genus *Sarcocystis*

The cysts are large bodies, up to half a millimeter or even more in diameter and are filled with narrow spores resembling more the sporozoites of a malaria parasite than those of *Sarcocystis* (Fig 6) They are easily distinguished from sarcocysts or other protozoa by their wide cyst wall. This is actually a double wall a narrow and nucleated inner wall and a thick and hyaline outer wall, up to 20μ in thickness.

E. *Toxoplasma gondii*

The classification and life history of *Toxoplasma* are as incompletely known as those of *Sarcocystis* in spite of its equally wide distribution in the animal kingdom. It is an important infection of laboratory animals in fact it was first described in domestic rabbits by Splendore (1909) in Brazil. It occurs commonly in dogs, cats, rodents sheep cattle, pigs, besides various wild mammals marsupials and birds (including chickens Numerous human cases have now been diagnosed in all continents and if positive serological reactions truly indicate past or present in

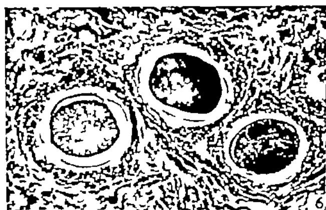












PLATE II

fection, then the number of human infections must run into millions.

In acute toxoplasmosis, the organism exists in the proliferative form as a crescentic body 4 or 5 μ long with a single nucleus in the chronic disease, the organisms are found in cysts of various size in different parts of the body In both types, muscle may be the site of infection (Fig 7) though much less commonly than is the brain. *Toxoplasma* is invariably intracellular at first and it invades a variety of cells including probably the muscle fiber But the origin of the focus in muscle is perhaps more commonly the lymphoid macrophage cell lying between the fibers than a muscle cell itself.

Whatever the origin, the skeletal and cardiac muscles not infrequently show parasites. Frenkel (1953) has demonstrated the existence of a severe myositis of the diaphragm in acute infections in mice and also cysts in skeletal and heart muscle in old-standing infections in pigeons, guinea pigs, etc. In man, a severe myositis may occur and Kass *et al* (1952) describe such a case in which a biopsy of the gastrocnemius muscle showed degeneration of individual muscle fibers surrounded by lymphocytes, plasma and mononuclear cells in a second biopsy the organism was found in the fibers. Later there was gross destruction of the muscle fibers, the sarcous content of the fiber becoming swollen, hyaline, and usually eosinophilic, with much fragmentation. The sarcolemmal nuclei first increased in number and size, then became hyperchromatic, and finally were shrunken and necrotic.

In the less acute cases, cysts may be present only in small numbers and be detected by accident, such as the single cyst found in the heart muscle of an American soldier drowned in the Panama Canal (Mantz *et al* 1949) Such infections, whether in man or animals often

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Fig. 6. *Besnoitia besnoiti* cysts in muscle of cow (Courtesy of Dr P L. Leroux.) Magnification × 93

Fig 7 Cysts of *Toxoplasma gondii* in thigh muscle of mouse (6 mo.-old infection) See also Lamson (1958) Magnification × 350

Fig. 8. Exoerythrocytic schizonts of *Plasmodium gallinaceum* in heart muscle of duck. Magnification × 1200

Fig. 9 Myocarditis in chick heart in *Plasmodium juxtanucleare* malaria. (Courtesy of Dr M. A. Al Dabagh.) Magnification × 350.

Fig. 10. *Leucocytozoon* megaloschizonts in heart muscle of bird. (Courtesy of Dr Cowan.) Magnification × 350.

Fig 11 Myxosporidian in heart muscle of sole. (Courtesy of Mr A. M. Qureshy) Magnification × 1000

give rise to difficulties in diagnosis, because the tissue for examination may well be inadequately prepared. If it is fresh, then diagnosis is easily made by inoculating a suspension into susceptible animals (e.g. the multimammate mouse) but a poorly stained section with cysts filled with small oval bodies might be mistaken for *Sarcocystis*, *Trypanosoma cruzi*, *Besnoitia*, or the "P" organism (Frenkel, 1956).

The type of myocarditis caused by *Toxoplasma* has been described by Bengtsson (1950): small cysts lie within the unchanged muscle fiber; larger ones are not so clearly inside, but in both types there is a complete absence of local inflammation. Foci of histiocytes, mast cells, and lymphocytes, however, are found scattered through the heart muscle. Potts and Williams (1956) described a fatal case in a man where autopsy revealed a much enlarged heart with foci of lymphocytes, plasma cells, and mononuclears occasionally perivascular but more often generalized in the heart muscle.

The transmission of *Toxoplasma* is still incompletely known and it is possible that it sometimes takes place by the consumption of infected meat. Lainson (1954) and others consider that the oral route of infection may be important in natural infections of dogs, cats, ferrets, or even man (Jacobs and Melton, 1958).

Congenital transmission of *Toxoplasma* is well established and in such cases the child is usually born dead or dies soon after birth. The muscles may show extensive involvement, particularly those of the heart. One of the earliest reports of human infection was made by Torres (1927) in Brazil: the newborn child showed permanent and generalized muscular contractures. At death two days later, sections of these muscles exhibited a condition of disseminated myositis, while the heart muscle was in a condition of acute myocarditis. All these lesions were associated with the presence of cysts. Nearly 30 years later, a whole series of such cases was described by Cardoso *et al.* (1956) from the same region.

F. Amoebic Abscess

The parenteral distribution of *Entamoeba histolytica* may be widespread in practically every organ and tissue of the human body, though sites other than the liver and lungs are only rarely affected. The amoeba is carried from the original site in the intestine by the bloodstream to a tissue or organ. There, perhaps because of some local damage, it starts to multiply, phagocytes accumulate and a small abscess forms. This abscess spreads by radial diffusion, the amoeba being numerous at

the advancing edge. Amoebic infection of the skin, subcutaneous tissue, or bone may extend into the neighboring muscles, e.g into the buttocks or thigh. A liver abscess due to this organism may rupture into the muscles of the diaphragm or into those of the anterior abdominal wall.

The ordinary amoebic ulcers of the large intestine develop in the submucosa and open into the lumen of the bowel sometimes, the organism progresses in the reverse direction and attacks the unstriated muscle, eventually reaching and penetrating the peritoneal coat of the intestine. Usually there is little tissue reaction in the muscularis in spite of a heavy infection of amoebae and extensive lysis of the muscle fibers.

G Malaria Parasites and Other Hemosporidia

Myocardial degeneration of the heart may occur in chronic malaria when repeated attacks of the disease lead to anemia and general debility Apart from this condition, a pernicious form of cardiac malaria occasionally develops in *Plasmodium falciparum* infections which usually has a rapidly fatal termination. In this species of malaria parasite, the growth of the parasite continues in the peripheral blood for only a short part of the cycle and final maturation takes place in the capillaries of the internal organs. The presence of sticky infected corpuscles in vessels of narrow caliber leads to a slowing down of the circulation of the blood in certain organs, including the heart. The endothelial lining of such vessels suffers, the cells swell, fluid passes from the blood into the adjacent muscle, and the processes of anoxemia and anoxia gradually increase Stasis, thrombosis, and hemorrhages through the weakened endothelium follow and large areas of cardiac muscle may be occupied by infarcts while flame-shaped hemorrhages occur on the pericardial surface (Garnham 1949)

Sections of the heart muscle from a fatal case of cardiac malaria show a characteristic picture. The smaller vessels are filled with erythrocytes containing mature pigmented schizonts of *P falciparum* together with numerous lymphoid macrophage cells the muscle cells themselves never contain parasites, but according to Maegraith (1948) small hemorrhages are occasionally found in the muscle substance around the small veins.

The condition is primarily a case of the parasites blocking the blood vessels of the muscle. The fibers become involved only secondarily Changes in the fibers usually occur as part of a fulminating process extending throughout the body The secondary changes include the

fatty degeneration of the muscle with a deposition of globules of irregular size clustered around the nucleus or distributed throughout the contractile substance. Similar changes are found in Rhesus monkeys dying of *P. knowlesi* malaria.

In avian malaria exoerythrocytic schizogony takes place in a great variety of tissue cells, chiefly, however, in those of the lymphoid macrophage type, including the endothelium of the smaller blood vessels. In *P. gallinaceum* infections of chickens, invasion of such cells may be very extensive: the skeletal muscles may become involved during the third week of infection, while the heart muscle is frequently the site of exoerythrocytic schizogony of this parasite (Fig. 8). In neither of these conditions is any marked cellular reaction visible among the occluded vessels, but the nutrition of the surrounding muscle must inevitably suffer. A similar involvement of the heart muscle occurs in other species of avian malaria, and in lizard malaria also, where the actual development of the parasite takes place in the connective tissue of the subendothelial layer of the heart.

It has long been known that chickens suffering from chronic *P. juxtanucleare* malaria develop a pericarditis with effusion from which they die, but it is only recently that Al Dabagh (1958) has demonstrated the nature of the pathological changes in the muscle. Large accumulations of adventitious cells, particularly lymphocytes, plasma cells, and macrophages, are present in between the muscle fibers of the heart, forming in places almost tumor-like masses. The muscle cells lose their striation and show other degenerative changes (Fig. 9).

Malaria parasites can also be found in the muscle of artificially infected mosquitoes during the sporogonic stage. Weathersby (1952) showed that when blood containing male and female gametocytes of *P. gallinaceum* is introduced into the hemocoele of a vector mosquito, fertilization follows and the oocysts grow to maturity principally between the muscle fibers of the thoracic muscle of the insect. Even in natural infections, sporozoites are found in large numbers among the muscle fibers. In neither instance does the presence of the parasite excite any visible cellular reaction.

Part of the exoerythrocytic cycle of *Leucocytozoon* takes place in the muscle cells of the heart. This hemosporidian has a cosmopolitan distribution in a wide variety of birds. In certain places, notably Canada (in ducks) and Scandinavia (in various game birds), the infection is of considerable veterinary importance. The birds become infected by the

bites of *Simulium* flies the sporozoites develop in different parts of the body including the heart muscle (Fig 10) The effect of the parasite on the host cell is quite extraordinary with the growth of the organism the nucleus and protoplasm of the host cell undergo an increase in size which can only be described as a case of gigantism. The host cell nucleus finally attains a diameter of 190 μ. *Leucocytozoon* is able to evoke this intense stimulation of growth in a variety of cells, probably chiefly of lymphoid macrophage origin, but Huff (1942) suggests, on the evidence of the resemblance of the shape of the megaloschizonts and their nuclei, that the parasites may develop in the muscle cell itself. The megaloschizont grows to a size of nearly half a millimeter and causes extensive damage to the heart muscle. This exoerythrocytic stage in the heart muscle is ephemeral and can only be detected very early in the infection. As in most protozoal infections, the presence of the unruptured parasites causes no reaction on the part of the host, but when the schizont bursts, a local infiltration of phagocytes takes place (Fallis *et al.*, 1956) The pathogenicity of *Leucocytozoon* in birds results, not so much from these lesions in the organs, but from the later heavy destruction of blood cells.

H MICROSPORIDIA AND MYXOSPORIDIA

Insects and fish are not uncommonly affected by protozoa belonging to the subclass Cnidosporidia, which possess spores of a unique character Well known examples are the microsporidians *Nosema bombycis* causing pébrine or silkworm disease, and *N apis* causing bee disease, and the myxosporidians affecting goldfish, trout, and other fish of commercial importance. In silkworm disease, colonies of the parasite invade muscle fibers under the cuticle of the insect, as well as most other tissues.

The muscle is, however not a very common site of development of these parasites, but a few species have a special affinity for this tissue Several genera of microsporidia attack the muscles of fish, frogs, and crustaceans and cause a hyaline degeneration of the muscle fibers (Grassé, 1953) *Glugea destruens* affects *Callionymus lyra G danilewskyi* affects the frog several species of *Thelohania* invade the muscles of fish and various invertebrates and cause their death. *Plistophora* attacks blennies and other fish, forming little protoplasmic masses between the fibrils, which develop into pansporoblasts.

An important myxosporidian parasite of fish is *Unicapsula muscularis*

which invades the skeletal muscle fibers of the halibut. After repeated multiplication of the parasite, the fiber becomes almost entirely obliterated and is occupied by a mass of spores. The condition is known as wormy halibut and is of some economic importance. It is found off the Pacific Coast of North America. *Myxobolus pfeifferi* is very fatal to species of *Barbus* (*B. fluviatilis*). It develops in the muscle of these fish and causes tumors up to 7 cm. in diameter. A fibrous corpuscle is formed by the host and inside this corpuscle the parasites continue their development with the formation of spores; eventually the parasites die and all that remains is a fibrous nodule containing many spores. *Myxobolus orbiculatus* attacks the muscles of other fish. Kudo (1954) has demonstrated a most unusual effect on muscle due to *Myxobolus intestinalis*. This organism invades the intestinal wall of the black crapple and causes rupture of some of the circular muscle fibers which then swivel round so as to lie at right angles to the main bundles, and project into the submucosa. *Myxosoma catostomi* is a parasite of the muscle and connective tissue of *Catostomus commersonii* (Fig. 11), while *Henneguya tegidiensis* becomes encapsulated in the muscles of the Welsh Gwyniad (Nicholas & Jones, 1959).

III. HELMINTHIC PARASITES

Unlike many of the protozoal infections of muscle, the helminthic infestations of this tissue are practically always part of a well-known cycle of the parasite, in which the exact stage and nature of the process are fully understood, even though the parasite may have undergone kaleidoscopic changes of host, and of tissue or organ in a single host.

Certain helminths are obligatory parasites of muscle, and the successful progress of their life history depends upon the consumption of meat. When man forms a link in this chain, the matter assumes public health importance, and meat inspection is a routine procedure in sanitary practice. The presence of cysticerci of tapeworms is particularly looked for; different countries have various standards for the treatment or disposal of infected carcases. Such procedures are well described in the monograph series of the World Health Organization (1957).

The prevention of trichinosis is also attempted in many countries by legal measures; thus in England, it is a legal obligation for pig farmers to cook all food given to pigs. The parasite in pork can be killed by adequate cooking, by rapid freezing to —35° C. or by irradiation with X- and γ-rays (Gould *et al.*, 1954).

A. Helminthic Parasites of the Muscle of Arthropods

Many species of filarial worms spend part of their developmental cycle in mosquitoes and other dipterous insects. The mosquito bites an infected individual and takes up the microfilaria either from the skin or the blood. The microfilariae in the course of 24 hours pass through the epithelial lining of the midgut of the insect into the hemocoele and thence to the muscles of the thorax. The larval forms of the parasite assume a typical sausage shape, measuring about 150 μ in length at first, but growing in the next few days to a length of 1 to 5 mm. or more (Fig 12) Lavoipierre (1958) and a few other workers have demonstrated that in some species at least, the filarial larvae after arrival between the fibers of the thoracic muscles, proceed to invade the fibers themselves, where they continue to grow The final maturation of the parasite takes place outside the muscle cell. While the thoracic muscles of arthropods are the muscles usually attacked by these parasites, a few species invade the leg muscles (*D vite* in ticks—Chabaud 1954) or the muscles of the abdomen or head. No evidence of marked tissue response is usually found, but in certain circumstances a reaction may occur if the species of intermediate host is the incorrect one, or if the temperature is abnormally raised or lowered, the larvae undergo a chitinous encapsulation (Kartman 1957) The chitin is apparently secreted by the tracheoles and hardens to form a thick capsule around the worm Manson Bahr (1912) describes such parasites as mummies lying in their coffins in the thoracic muscles of *Stegomyia* mosquitoes

Development of the worm in the muscles of insects is strikingly similar in all the different species or genera of filaria and in the different insect hosts. Lavoipierre (1958) gives a complete list of arthropods in whose muscles filarial larvae undergo development. This information is presented in Table I

B Cysticercosis and Other Cestode Infections of Muscle

One of the commonest parasitic infections of muscle is the larval stage of various species of tapeworms, which in their adult form usually live in the intestine of another host. The eggs pass out in the feces and are ingested by the intermediate host, a herbivorous or omnivorous vertebrate animal on hatching the embryos penetrate the intestinal wall and settle down in the muscles, which they reach via the bloodstream or lymphatics In the muscle the larva undergoes a meta

of the whole lesion will ensue. The calcified bodies are easily seen in skiagrams of the muscles.

Taenia solium in the muscles of the pig has been known from the time of Aristophanes (450 B.C.). Today, the infection is widespread throughout the world, except in Mohammedan or Jewish lands where pork is not eaten. The larva is known as *Cysticercus cellulosae* and is a hyaline or opalescent body up to 15 mm. long and 8 mm. wide, containing the invaginated scolex (Fig. 13). These bodies are very long lived and may remain viable for 25 years. The following muscles, in order of frequency, are affected: Heart, intercostals, neck and shoulder, diaphragm, and tongue. Man is normally the definitive host of the tapeworm, but he occasionally acts as the intermediate host also, when the larval form develops in the muscles and in other tissues. In the skeletal muscle, its presence is usually well tolerated, unless the infestation is heavy, when muscular fatigue, cramps, and general lassitude result. In the heart, the parasite provokes a myocarditis and the aortic and mitral valves become affected.

Taenia saginata is even more widespread in its distribution, the intermediate host being cattle. In Europe, this species of tapeworm is seven times as common as *T. solium* (Brumpt, 1949). *Cysticercus bovis* has the same appearance as *C. cellulosae* but is slightly smaller in size (Fig. 14); it occurs in antelopes, giraffe, llamas, and other bovids, while man is the definitive host of this cestode also. In cattle, the following muscles are particularly affected: heart, masticatory muscles, tongue, and diaphragm (in the ratio of 8 : 4 : 1 : 1).

The larval stages of other cestodes are only rarely located in muscle. The coenurus of *Multiceps serialis* is occasionally found in the muscles of the back of man. Small foci of this infection exist, e.g. on the slopes of Mount Kenya. The coenurus is a cystlike structure characterized by the possession of numerous scolices.

The hydatid cyst of *Echinococcus granulosus* is occasionally found in the heart and skeletal muscles of man and other mammals. Infection takes place when the animal swallows the eggs of this tapeworm, whose adult habitat is the small intestine of dogs, jackals, and wolves. The adult worm is tiny, but the larva (the hydatid cyst) is enormously larger and is characterized by having a laminated layer and a germinal layer from which issue brood capsules, each containing many scolices. *Echinococcus granulosus* is particularly common in Iceland, Canada, Argentina, South Africa, and South Australia. The large size of the cyst interferes

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Malaria parasites can also be found in the muscle of artificially infected mosquitoes during the sporogonic stage. Weathersby (1952) showed that when blood containing male and female gametocytes of *P. gallinaceum* is introduced into the hemocoele of a vector mosquito, fertilization follows and the oocysts grow to maturity principally between the muscle fibers of the thoracic muscle of the insect. Even in natural infections, sporozoites are found in large numbers among the muscle fibers. In neither instance does the presence of the parasite excite any visible cellular reaction.

Part of the exoerythrocytic cycle of *Leucocytozoon* takes place in the muscle cells of the heart. This hemosporidian has a cosmopolitan distribution in a wide variety of birds. In certain places, notably Canada (in ducks) and Scandinavia (in various game birds), the infection is of considerable veterinary importance. The birds become infected by the

bites of *Simulium* flies the sporozoites develop in different parts of the body including the heart muscle (Fig 10) The effect of the parasite on the host cell is quite extraordinary with the growth of the organism the nucleus and protoplasm of the host cell undergo an increase in size which can only be described as a case of gigantism. The host cell nucleus finally attains a diameter of 190 μ. *Leucocytozoon* is able to evoke this intense stimulation of growth in a variety of cells, probably chiefly of lymphoid macrophage origin, but Huff (1942) suggests, on the evidence of the resemblance of the shape of the megaloschizonts and their nuclei that the parasites may develop in the muscle cell itself. The megaloschizont grows to a size of nearly half a millimeter and causes extensive damage to the heart muscle. This exoerythrocytic stage in the heart muscle is ephemeral and can only be detected very early in the infection. As in most protozoal infections, the presence of the unruptured parasites causes no reaction on the part of the host, but when the schizont bursts, a local infiltration of phagocytes takes place (Fallis *et al.*, 1956) The pathogenicity of *Leucocytozoon* in birds results, not so much from these lesions in the organs, but from the later heavy destruction of blood cells.

H Microsporidia and Myxosporidia

Insects and fish are not uncommonly affected by protozoa belonging to the subclass Cnidosporidia, which possess spores of a unique character Well-known examples are the microsporidians *Nosema bombycis* causing pébrine or silkworm disease, and *N apis* causing bee disease, and the myxosporidians affecting goldfish trout, and other fish of commercial importance. In silkworm disease, colonies of the parasite invade muscle fibers under the cuticle of the insect, as well as most other tissues.

The muscle is, however not a very common site of development of these parasites, but a few species have a special affinity for this tissue. Several genera of microsporidia attack the muscles of fish, frogs, and crustaceans and cause a hyaline degeneration of the muscle fibers (Grassé, 1953) *Glugea destruens* affects *Callionymus lyra* *G danilewskyi* affects the frog several species of *Thelohania* invade the muscles of fish and various invertebrates and cause their death. *Plistophora* attacks blennies and other fish forming little protoplasmic masses between the fibrils, which develop into pansporoblasts.

An important myxosporidian parasite of fish is *Unicapsula muscularis*

fatty degeneration of the muscle with a deposition of globules of irregular size clustered around the nucleus or distributed throughout the contractile substance. Similar changes are found in Rhesus monkeys dying of *P knowlesi* malaria.

In avian malaria exoerythrocytic schizogony takes place in a great variety of tissue cells, chiefly however in those of the lymphoid macrophage type, including the endothelium of the smaller blood vessels In *P gallinaceum* infections of chickens invasion of such cells may be very extensive the skeletal muscles may become involved during the third week of infection while the heart muscle is frequently the site of exoerythrocytic schizogony of this parasite (Fig 8) In neither of these conditions is any marked cellular reaction visible among the occluded vessels, but the nutrition of the surrounding muscle must inevitably suffer A similar involvement of the heart muscle occurs in other species of avian malaria and in lizard malaria also where the actual development of the parasite takes place in the connective tissue of the subendothelial layer of the heart.

It has long been known that chickens suffering from chronic *P juxtanucleare* malaria develop a pericarditis with effusion from which they die, but it is only recently that Al Dabagh (1958) has demonstrated the nature of the pathological changes in the muscle. Large accumulations of adventitious cells, particularly lymphocytes plasma cells, and macrophages, are present in between the muscle fibers of the heart, forming in places almost tumor like masses. The muscle cells lose their striation and show other degenerative changes (Fig 9)

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A. Helminthic Parasites of the Muscle of Arthropods

Many species of filarial worms spend part of their developmental cycle in mosquitoes and other dipterous insects. The mosquito bites an infected individual and takes up the microfilaria either from the skin or the blood The microfilariae in the course of 24 hours pass through the epithelial lining of the midgut of the insect into the hemocoele and thence to the muscles of the thorax. The larval forms of the parasite assume a typical sausage shape, measuring about 150 μ in length at first, but growing in the next few days to a length of 1 to 5 mm or more (Fig 12) Lavoipierre (1958) and a few other workers have demonstrated that in some species at least, the filarial larvae after arrival between the fibers of the thoracic muscles, proceed to invade the fibers themselves, where they continue to grow The final maturation of the parasite takes place outside the muscle cell While the thoracic muscles of arthropods are the muscles usually attacked by these parasites, a few species invade the leg muscles (*D vitæ* in ticks—Chabaud 1954) or the muscles of the abdomen or head. No evidence of marked tissue response is usually found, but in certain circumstances a reaction may occur· if the species of intermediate host is the incorrect one, or if the temperature is abnormally raised or lowered, the larvae undergo a chitinous encapsulation (Kartman 1957) The chitin is apparently secreted by the tracheoles, and hardens to form a thick capsule around the worm Manson-Bahr (1912) describes such parasites as mummies lying in their coffins in the thoracic muscles of *Stegomyia* mosquitoes.

Development of the worm in the muscles of insects is strikingly similar in all the different species or genera of filaria and in the different insect hosts. Lavoipierre (1958) gives a complete list of arthropods in whose muscles filarial larvae undergo development. This information is presented in Table I

B Cysticercosis and Other Cestode Infections of Muscle

One of the commonest parasitic infections of muscle is the larval stage of various species of tapeworms, which in their adult form usually live in the intestine of another host. The eggs pass out in the feces and are ingested by the intermediate host, a herbivorous or omnivorous vertebrate animal on hatching the embryos penetrate the intestinal wall and settle down in the muscles, which they reach via the bloodstream or lymphatics. In the muscle, the larva undergoes a meta

which invades the skeletal muscle fibers of the halibut. After repeated multiplication of the parasite, the fiber becomes almost entirely obliterated and is occupied by a mass of spores. The condition is known as wormy halibut and is of some economic importance. It is found off the Pacific Coast of North America. *Myxobolus pfeifferi* is very fatal to species of *Barbus* (*B. fluviatilis*). It develops in the muscle of these fish and causes tumors up to 7 cm. in diameter. A fibrous corpuscle is formed by the host and inside this corpuscle the parasites continue their development, with the formation of spores; eventually the parasites die and all that remains is a fibrous nodule containing many spores. *Myxobolus orbiculatus* attacks the muscles of other fish. Kudo (1954) has demonstrated a most unusual effect on muscle due to *Myxobolus intestinalis*. This organism invades the intestinal wall of the black crappie and causes rupture of some of the circular muscle fibers which then swivel round so as to lie at right angles to the main bundles, and project into the submucosa. *Myxosoma catostomi* is a parasite of the muscle and connective tissue of *Catostomus commersonii* (Fig. 11), while *Henneguya tegidiensis* becomes encapsulated in the muscles of the Welsh Gwyniad (Nicholas & Jones, 1959).

III. HELMINTHIC PARASITES

Unlike many of the protozoal infections of muscle, the helminthic infestations of this tissue are practically always part of a well-known cycle of the parasite, in which the exact stage and nature of the process are fully understood, even though the parasite may have undergone kaleidoscopic changes of host and of tissue or organ in a single host.

Certain helminths are obligatory parasites of muscle, and the successful progress of their life history depends upon the consumption of meat. When man forms a link in this chain, the matter assumes public health importance, and meat inspection is a routine procedure in sanitary practice. The presence of cysticerci of tapeworms is particularly looked for; different countries have various standards for the treatment or disposal of infected carcasses. Such procedures are well described in the monograph series of the World Health Organization (1957).

The prevention of trichinosis is also attempted in many countries by legal measures; thus in England, it is a legal obligation for pig farmers to cook all food given to pigs. The parasite in pork can be killed by adequate cooking, by rapid freezing to —35° C. or by irradiation with X- and γ-rays (Gould *et al.*, 1954).

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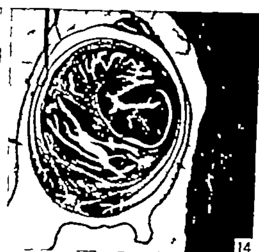






PLATE III

TABLE I

ARTHROPODS WHOSE MUSCLES HARBOUR VARIOUS FILARIAL LARVAE

Arthropod	Parasite and Primary Host
Culex fatigans	*Conispiculum flavescens* of the lizard
Culicoides species	*Dipetalonema perstans* of man
Culicoides species	*Dipetalonema streptocerca* of man
Culicoides furens	*Mansonella ozzardi* of man
Culicoides nubeculosus	*Onchocerca cervicalis* of horses
Culicoides pungens	*Onchocerca gibsoni* of cattle
Ornithodoros tartakovskyi	*Dipetalonema vitae* of merion
Forcipomyia velox	*Icosiella neglecta* of frog
Simulium ornatum	*Onchocerca gutturosa* of cattle
Simulium species	*Onchocerca volvulus* of man
Anopheles sinensis and others	*Setaria digitata* of sheep, goats, horses
Aedes and other mosquitoes	*Wuchereria bancrofti*, etc. of man
Chrysops species	*Loa loa* of man

morphosis into a cysticercus or other bladderlike structure, according to the genus of tapeworm. The life cycle is completed when the meat is consumed by the definitive host, in whom the cysticercus is digested out of the muscle. The uninjured head of the worm escapes into the small intestine to give rise to a mature tapeworm.

Various degrees of infestation of muscle occur, from a single cysticercus to a condition where all the skeletal muscles of the body, together with the tongue and heart, are honeycombed with the parasite. Such infected meat in the case of the pig is known popularly as "measly pork." It is probable that the development of this parasite in the muscle is extracellular in the adipose connective tissue between the fibers; as it grows, it pushes aside the fibers, although the lateral pressure tends to make it assume an ovoid form. A tissue reaction always accompanies the formation of the cysticercus, whose outer wall is the product of the host. Particularly in abnormal hosts, such as cysticercosis in man, a further reaction is likely to occur. Leucocytes, eosinophiles, and cholesterol crystals may accumulate; finally calcification

FIG. 12. Larvae of *Mansonella ozzardi* in thoracic muscle of *Culicoides* sp. (Courtesy of Professor J. J. C. Buckley.) Magnification × 75.

FIG. 13. *Cysticercus cellulosae* in muscle of pig. Magnification × 17.

FIG. 14. *Cysticercus bovis* in muscle of ox. Magnification × 20.

FIG. 15. Larvae of *Diphyllobothrium* sp. in muscle of turbot. Magnification × 60.

of the whole lesion will ensue. The calcified bodies are easily seen in skiagrams of the muscles

Taenia solium in the muscles of the pig has been known from the time of Aristophanes (450 B C.) Today the infection is widespread throughout the world, except in Mohammedan or Jewish lands where pork is not eaten. The larva is known as *Cysticercus cellulosae* and is a hyaline or opalescent body up to 15 mm. long and 8 mm. wide, containing the invaginated scolex (Fig 13) These bodies are very long-lived and may remain viable for 25 years. The following muscles, in order of frequency are affected Heart, intercostals, neck and shoulder, diaphragm, and tongue. Man is normally the definitive host of the tapeworm, but he occasionally acts as the intermediate host also, when the larval form develops in the muscles and in other tissues In the skeletal muscle, its presence is usually well tolerated, unless the infestation is heavy when muscular fatigue, cramps, and general lassitude result. In the heart, the parasite provokes a myocarditis and the aortic and mitral valves become affected.

Taenia saginata is even more widespread in its distribution, the intermediate host being cattle In Europe, this species of tapeworm is seven times as common as *T solium* (Brumpt, 1949) *Cysticercus bovis* has the same appearance as *C cellulosae* but is slightly smaller in size (Fig 14) it occurs in antelopes, giraffe, llamas and other bovids, while man is the definitive host of this cestode also In cattle, the following muscles are particularly affected heart, masticatory muscles, tongue, and diaphragm (in the ratio of 8 4 1 1)

The larval stages of other cestodes are only rarely located in muscle. The coenurus of *Multiceps serialis* is occasionally found in the muscles of the back of man. Small foci of this infection exist, e.g on the slopes of Mount Kenya. The coenurus is a cystlike structure characterized by the possession of numerous scolices.

The hydatid cyst of *Echinococcus granulosus* is occasionally found in the heart and skeletal muscles of man and other mammals. Infection takes place when the animal swallows the eggs of this tapeworm whose adult habitat is the small intestine of dogs, jackals, and wolves. The adult worm is tiny but the larva (the hydatid cyst) is enormously larger and is characterized by having a laminated layer and a germinal layer from which issue brood capsules, each containing many scolices. *Echinococcus granulosus* is particularly common in Iceland, Canada, Argentina, South Africa, and South Australia. The large size of the cyst interferes

with the cardiac rhythm and it may even rupture out of the muscle into one of the chambers of the heart in the skeletal muscle its pathogenicity is confined to pressure changes.

A different type of cestode invasion of muscle is seen in the immature stages of *Diphyllobothrium* in the muscles of fish (Fig 15) The mature worm lives in the intestine of man dogs cats, foxes, bears and other animals the eggs pass out in the feces a coracidium hatches in water and is swallowed by a *Cyclops* where it becomes a procercoid and the *Cyclops* is eaten by a freshwater fish, in whose muscles the parasite continues its development as a plerocercoid or sparganum. Man becomes infected by eating uncooked or improperly cooked fish. The following fish are commonly found infected pike, Miller's thumb perch salmon, trout, grayling and barbel. The infection is heaviest in the older fish The plerocercoid is easily visible to the naked eye, measuring up to 2 cm. in length, and is usually encapsulated. A fibrous wall is formed as a result of a tissue response by the host. In other respects, the muscle is unaffected and these plerocercoids seem to be harmless to the fish. In man, this stage of *D mansoni* (the sparganum) is rarely found, but according to Faust (1949) the spargana multiply in the muscle fascia and elsewhere, and the infected area becomes edematous and painful.

C. Trichinosis

Trichinella spiralis is an extremely common parasite of the muscles of man, cat, rat, dog pig bear mongoose, and other animals, and is of cosmopolitan distribution. Man contracts the infection by eating uncooked meat. The consumption of pork sausages is a particularly common method of infection. In some places [e.g in the Liverpool outbreak described by Semple *et al* (1954)] the incidence of the infection is much higher in women than men. This is because women in these localities have the habit of eating raw sausage meat In men, occupation is an important factor such as the handling of infected pork by slaughter house workers or butchers.

The adult nematode lives in the mucosa of the small intestine embryos are evacuated and migrate by the lymphatics or portal vein to the musculature of the body particularly the diaphragm, intercostals, and the muscles of the throat, tongue and eye in the skeletal muscles the parasites tend to congregate towards the tendinous extremities.

Encystment of the larva follows (Fig 16) though the exact location

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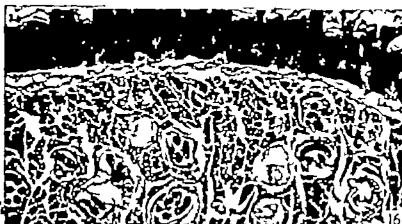








PLATE IV

of this process is still debatable. It was originally thought that the larva penetrated the muscle cell, where an ellipsoidal cyst capsule was quickly formed, but the French school (Brumpt, 1949) have demonstrated that the larva inhabits the interfascicular connective tissue from which the cyst wall is derived

The cysts are just visible to the naked eye as lemon-shaped bodies about half a millimeter in length. The long axis lies in the direction of the fibers, and the larva is tightly coiled up inside the cyst. The cysts remain viable for many years and have been found in man over 30 years after infection. Calcification of the older cysts frequently takes place.

The effect of the parasite depends upon the intensity of the infestation. If this is high, grave symptoms follow including high fever and delirium, accompanied by severe muscle pains, difficulty in swallowing eating, and breathing and ending in cachexia and death between the second and seventh week. In light infections, the symptoms gradually decline and the patient passes into a state of premunition.

According to Faust (1949) the histological changes in the muscle are quite characteristic. the muscle fibers around the cysts lose their striae and the nucleus divides repeatedly· adjacent fibers swell and the connective tissue increases in amount. This hyperplasia continues at the expense of the muscle fibers, which gradually degenerate. The removal by biopsy of a small portion of deltoid or biceps muscle from near the tendinous attachment and the examination in a trichina press under low magnification will usually reveal the presence of the organism. Digestion of a portion of muscle is recommended for the diagnosis of a suspected clinical infection small fragments are incubated for some hours with 1% acid pepsin the mixture is then centrifuged and the deposit is examined on an inverted microscope, in a hollow ground slide the heavier larvae are then easily visible in the bottom of the cell.

FIG. 16. *Trichinella spiralis* larvae in tongue of experimentally infected rat. Magnification × 85.

FIG. 17 Metacercariae of *Opisthorchis felineus* in muscle of fish. (Crush preparation.) Magnification × 75.

FIG. 18. *Heterophyes heterophyes* egg in human heart muscle with myocarditis. Magnification × 300.

FIG. 19 *Onchocerca* nodule in muscle of cow (Courtesy of Dr P L. Leroux.) Magnification × 22.

Myocarditis due to *Trichinella spiralis* is common an interstitial infiltrate composed of eosinophiles and plasma cells accumulates beneath the pericardium, while granulomata, composed of histiocytes and giant cells surrounded by various adventitious cells, are found deeper in the myocardium (Ash and Spitz, 1945)

An outbreak of human trichinosis in Liverpool in 1953 accompanied by two fatalities, provided an opportunity to study the pathology of the disease in some detail Kershaw *et al* (1956) describe the distribution of the larvae in the different muscles of the body on a quantitative basis (number of larvae per gram of muscle) and showed that in a heavy infection the muscles of the tongue, diaphragm, forearm, and calf were the most severely affected, whereas in a light infection, the infection was most intense in the limb muscles and absent in the tongue. The practice, therefore, of confining trichinoscopy to the diaphragm is unwise, and the somatic musculature should be included also Paradoxically the degree of pathogenicity of the organism is proportional to the resistance offered by the host. In the usual infections, the larvae encyst harmlessly in the skeletal muscles in a fatal case, the larvae go to the heart muscle where a widespread focal destruction of fibers follows the intense inflammatory reaction, with disintegration of the parasite (Semple *et al.*, 1954)

D Helminthic Parasites of Muscles of Fish and Crustacea

Fish are affected by a large variety of helminths of all types. The plerocercoid stage of *Diphyllobothrium* has been described above. Immature stages of various flukes are found in the musculature of fish. The metacercariae of *Opisthorchis felineus* occur in the tench (Fig 17) In the common human fluke of the Far East, *Clonorchis sinensis* the cercariae attack the fish (particularly the red fish *Carassius auratus*) and get through the scales and into the flesh where they encyst as metacercariae. A double capsule is formed in the muscle, the outer one being a tissue response by the host. Raw fish are eaten the cyst walls are dissolved in the duodenum and the parasite continues its development in man, dogs, cats, badgers, mink, and other animals. A similar type of development occurs in the small fluke *Heterophyes heterophyes* The metacercariae of this helminth encyst in between the muscle fibers of various fish, including the mullet. The adult fluke lives normally in the intestine of man and other mammals occasionally it migrates from this site to the heart muscle (Fig 18) where eggs are deposited, and a

myocarditis develops, with edema, hemorrhage, and myocardial degeneration ending in death.

Another fluke, *Paragonimus westermani* spends part of its developmental stage in the muscle and other tissues of crabs and crayfish The metacercariae become encysted in the muscles of the thoracic legs and in the cephalothorax itself. The cysts are pearly white and lie encapsulated in host tissue envelopes and derive nourishment from the host (Faust and Russell, 1958) The cyst is fixed to the muscle by a special fibrous attachment present at one end of the wall. Man becomes infected by eating half pickled crustaceans, in which the worm is still alive. Normally the fluke makes its way to the lungs of the human host, but sometimes it loses its way and settles down in one of the skeletal muscles instead.

Larval nematodes may be found in large numbers in the muscles of a variety of sea water fish Thus, the flesh of cod off the coast of Iceland is often found to be heavily infested with a small threadworm (Syme, 1949) The larva of different species of *Anisakis* may be found in the flesh of both freshwater and marine fishes while the larvae of *Porrocaecum* and *Anisakis* are common in thin walled cysts in the musculature of Icelandic cods (Grainger 1959) No gross damage appears to be produced by these worms, though in the case of cestodes, Linton (1908) noted that the American butterfish when heavily parasitized with the cysts weighed much less than uninfected specimens however, he found no evidence of any inflammatory or pathological condition of the muscle.

E. Nematode Parasites of Muscle

The nematodes form one of the largest classes in the animal kingdom and it is therefore not surprising to find them well represented as parasites of muscle *Trichinella* in man, filaria in mice, and larval nematodes in fish have been considered separately but many more examples exist and two more are mentioned here to illustrate larval and adult stages respectively

The round worm, *Ascaris devosi* lives in its adult form in the intestine of Viverridae its development in an intermediate host, the white mouse has been studied by Sprent (1952), who showed that unlike the human species, this worm migrated in large numbers via the portal system into the general musculature, particularly in the anterior part of the body A week after infection, the parasite may become encapsuled

in the diaphragm or heart, after 3 weeks white nodules imprisoning the third stage larvae are very conspicuous in the somatic musculature. The nodules consist of a layer of fibroblasts, histocytes, and inflammatory cells around the parasite. The ferret or other carnivorous mammal eats such an infected mouse and the larvae are set free in the intestine, where they grow to maturity

Onchocerca is found in various species of mammals including man and in most animals, the adult form of the worm is enclosed in nodules lying in the subcutaneous tissue. In eland and cattle of tropical and South Africa, a species of *Onchocerca* is known (Leroux, 1947) which inhabits nodules projecting deeply into the muscles of the trunk of these animals (Fig 19) In sections of such structures, the adult filarial worms are seen lying in a cyst surrounded by dense fibrous tissue compressing the muscle fibers in the vicinity The tissues may be riddled with these nodules, and the meat is of inferior quality but no specific lesions in the muscle are to be found Another species of *Onchocerca* occurs in the tendinous insertion of the triceps muscle of antelopes in African swamps.

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CHAPTER V

Muscle Regeneration and Repair

E. J. FIELD

I. HISTORICAL INTRODUCTION

Early investigations of the repair and regeneration of muscle tissue date from the second half of the eighteenth and the beginning of the nineteenth century when the process was studied with the naked eye in dogs, cats, and rabbits. From these studies, it emerged that destructive lesions in muscle resulted in fibrous scarring. Despite the observations of Nannoni (1782), Zimmerman (1812), and Guensberg (1848) who first claimed that muscle was capable of regeneration after damage, this teaching was long accepted and indeed, as will appear, contains a good deal of truth.

The early literature has been summarized by Kuttner and Landois

(1913) During the second half of the nineteenth century, a number of well worked out papers appeared in which regenerative capacity of striated muscle was clearly recognized though different views were taken of the details of the process. Allowing for the undeveloped state of biological technique at the time, the reader today is astonished at the accuracy of many of the observations made, and the shrewdness with which they were interpreted. For example, as early as 1858, Bottcher noted the nuclear proliferation which occurred in the first 24 hours at the margins of a muscle wound in the frog, rabbit, and rat. Weber (*1863 1867*) using dogs, cats, and rabbits, concluded that new muscle cells were derived from old surviving ones, and it is of considerable interest to find in Weber's later paper (1867 p 236) a suggestion of the "budding" theory usually attributed to Neumann (1868) "Zuweilen schiebt sich aus dem abgerissenen Ende eines Bündels innerhalb des konisch zulaufenden Sarkolemmschlauches oder vor das abgerissene Ende desselben hervortretend, ein schmaler blasser fein gestreifter *Streifen kontraktilen Protoplasmas* hervor den man als neugebildet ansehen muss, da er von der alten quergestreiften Substanz sich deutlich abgrenzt." The correspondence of this description with the modern view as will appear below is striking Another completely modern idea was brought forward by Aufrecht (1868) from his study of experimental wounds in rabbits and guinea pigs when he suggested that the condition of the sarcolemma was of paramount *importance for regeneration.* Waldeyer (*1865*) described the formation of "Sarkolemmschläuche" during muscle regeneration and Neumann (1868) introduced the term "Muskelknospen" to describe the buds which he found growing from the ends of surviving muscle fibers.

About this time, a curious thesis was put forward (Maslowsky *1868*) namely that new muscle cells could be formed from the leucocytes of the blood—a theory developed apparently under the all pervading influence of the Cohnheim School.

Much experimental work was carried out, and in 1893 Volkmann's monumental paper appeared—"Ueber die Regeneration des quer gestreiften Muskelgewebes beim Menschen und Säugethier"— in *which the process of regeneration and repair was exhaustively examined* in human, rabbit, and guinea pig material under a variety of con ditions which he grouped into those which spared the sarcolemma and those in which it was destroyed Meanwhile, Zenker (1864) had given his classic description of the waxy degeneration commonly occurring in

typhoid fever ("Typhus abdominalis" of German authors) and this stimulated much study of the regeneration which was found to take place so completely in this condition (Hoffmann 1868 Janowitsch Tschainski, 1869 1870) A further notable contribution was made by Kuttner and Landois (1913) while the most recent detailed studies have been made by Le Gros Clark (1946) and Godman (1957)

In general it may be said that modern studies have confirmed and extended many of the early observations, especially those of Aufrecht and Volkmann noted above, and they have also emphasized the predominant importance in higher vertebrates of regeneration by "budding" from old surviving muscle fibers.

II. FACTORS AFFECTING DEGREE OF MUSCLE FIBER REGENERATION

A. Survival and Vitality of Adjacent Muscle Fibers

The regeneration of striated muscle fibers after injury varies greatly in extent and effectiveness. It can lead to complete restitution provided that the fibers which are to make up the deficit are in good condition i e they retain their vitality and have adequate nutrition. Obviously the general nutritional condition of the body will be of importance as will also any disturbances of local circulation, superadded infection, or any other condition which might lead to local proliferation of connective tissue (e.g the presence of a foreign body) Any factor which leads to the excessive production of fibrous tissue militates against good muscle fiber regeneration The harmful effect seems to be very largely a mechanical one but it may be in part the result of competition for the available nutrition in the region

B Condition of the Sarcolemma of the Damaged Fibers Nature of the Primary Injury

Other things being equal a great deal depends upon the preservation of the sarcolemma after the injury Survival of the sheaths is a prerequisite for complete reconstitution in mammals, though apparently not in fishes.

It follows from this that regenerative changes vary with the initial causation of the damage in so far as this exercises an effect upon the sarcolemma Thus at one end of the scale we have Zenker's waxy degeneration which leaves the sarcolemma intact and results in complete restitution of muscle fibers while at the other end total destruc-

tion of muscle fibers and sarcolemmal sheaths results in imperfect and frustrated attempts at regeneration. As will be seen below (Section VIII) absence of the sarcolemma has been associated with the observed failure of heart muscle to regenerate.

C. Species Differences

A thorough investigation of regenerative changes in a series of different vertebrates was undertaken by Schminke (1907 1908 1909)

1 *Ichthyopsida*

In ichthyopsida (fishes and amphibians) Schminke found new formation of muscle fibers to take place "from the elements of the old fibres" by terminal outgrowth of buds, i.e. regeneration to take place "in continuity" with the old fibers. Only in Triton (a tailed amphibian) did Schminke find the process to be different regeneration did not take place in continuity with surviving fibers bordering the damaged region, but originated from isolated sarcoblasts, i.e. by a "discontinuous" process. The same observation was made in the salamander (*S maculata*) by Galeotti and Levi (1893)

2. *Sauropsida*

In sauropsida (reptiles and birds) regeneration was again found to be by the "continuous" process. However in the legless reptile *Anguis fragilis* (the blindworm) both "continuous" and "discontinuous" formation of new muscle fibers was seen. The latter occurred especially where a muscle fiber had disappeared and left behind only a chain of nuclei, each surrounded by a small rim of cytoplasm

3 *Mammals*

In mammals, Schminke found the regenerative process to be essentially by the "continuous" method. It is possible, however that minor species differences may occur and be responsible, perhaps, for the conflicting opinions expressed by different authors (von Meyenburg 1929)

III. ZENKER'S WAXY DEGENERATION (HYALINE DEGENERATION)

A detailed description of Zenker's degeneration will be given here because it is the human condition, par excellence, in which muscle fiber regeneration may take place in full (in the absence of compli-

cations) and it illustrates very well the conditions necessary for such complete regeneration. According to Hoffmann (1868) Gendrin (1832) was the first to note the "fish-flesh" appearance of muscles in subjects dying of cholera. While other workers made sundry observations in various other infective conditions (e.g Virchow 1852, 1857), it was in Zenker's monograph that the first full examination of the change which has since come to be associated with his name was published (Zenker 1864) Zenker worked on material obtained during the great typhoid epidemic in Dresden between the years 1859 and 1862 and described the changes in a large series of cases, especially in the adductor muscles of the thigh and in the rectus abdominis muscle, though many other muscles might be affected—even the tensor tympani and the laryngeal muscles. Indeed, involvement of the diaphragm has been though to be a factor in determining a fatal outcome in some cases of lobar pneumonia (Wells, 1927)

It is, of course now well recognized that Zenker's degeneration is by no means specific to typhoid fever but may be an incidental finding in a variety of other infections both acute and chronic [e.g tetanus, typhus fever puerperal fever miliary tuberculosis, diphteria, scarlet fever influenza, (Wolbach and Frothingham, 1923) etc.] as well as in such nonspecific toxic conditions as kernicterus neonatorum (Beneke, 1912) and even epilepsy (Stemmler 1914)

A. Macroscopic Appearances

Only regions in which the change is extensive can be recognized by the naked eye with the microscope, changes are found to be much more widespread Affected areas are pale, either pink or yellowish grey depending on the degree of the lesion, and were described by Zenker as resembling "fish flesh." The transition to adjacent sound muscle is gradual The increased thickness of an affected muscle is often striking especially on longitudinal section. Affected muscle is brittle and dry Later it undergoes diminution in volume and ultimately complete regeneration, as a rule.

While there does not seem to be any specific clinical symptomatology associated with Zenker's degeneration, which is essentially a post mortem finding it may occasionally lead to muscle rupture and hematoma formation (Virchow 1857)

B. Microscopic Appearances

1. *Degeneration Changes*

The microscopical changes are *essentially focal and often limited to* a segment of muscle fiber.

Proliferation of sarcolemmal nuclei in the affected segment is an early finding. According to Volkmann (1893), this may indeed be the earliest sign, preceding changes in sarcoplasm and myofibrils. This finding was confirmed by Forbus (1926a) who relates the sequence of changes to the acuteness and intensity of the causal toxemia. A gradual onset manifests itself by well marked proliferation of sarcolemmal nuclei, while an overwhelming poisoning may lead to obvious degenerative changes in the cytoplasm before the nuclear response has occurred.

The nuclei enlarge slightly, elongate, and divide amitotically to produce a line of nuclei, often contiguous. A clear cytoplasmic area surrounding them is prominent.

Slight granular clouding and swelling of the sarcoplasm occurs, so that cross striations become blurred and obscured over the affected segments. *Longitudinal striations tend to become irregular and accen*tuated and the whole affected segment is swollen, sometimes to twice its normal thickness. Accumulation of degeneration products may lead to the formation of small vesicles and fat droplets. The changes are characteristically discontinuous along the course of a given muscle fiber, so that a substantially altered segment may be interposed between portions of normal fiber. The altered sarcoplasm is gradually converted over the space of about 2 or 3 weeks into a homogeneous eosinophilic, highly refractile mass, contained within an intact sarcolemmal membrane continued at either end into normal sarcolemma.

A condition which sometimes appears early is Bowman's "discoidal" or "conchoidal" degeneration ("schollige Zerklüpfung" of German writers) (Adams *et al.*, 1953), though it was not recorded by Forbus (1926a) in his detailed account of the histology of the condition. In discoidal degeneration, the muscle fibers show a marked tendency to fragment across the Z lines, sometimes in a zig-zag manner. The appearances first described by Bowman (1840) are by no means limited to the Zenker type of degeneration, but are also seen in other varieties, *for example*, as a consequence of ischemia (Le Gros Clark, 1946). Indeed, the whole sequence of changes which culminates in

Zenker's degeneration may be brought about by several different noxious agencies, as will appear below

Sometimes, a well marked longitudinal splitting of a degenerating muscle fiber may occur and it is possible that such appearances may have been responsible for the theory that regeneration could take place in this way (Neumann, 1868 Nauwerck, 1890 Forbus, 1926a) Such differences in interpretation are notoriously difficult if indeed possible to resolve when based solely on static microscopic snapshots of different stages in an evolving dynamic process.

The toxic destruction of sarcoplasm and myofibrils in Zenker's degeneration leaves the sarcolemma and endomysium intact and this feature is the key to the very high degree of restitution which later follows.

2 *Regeneration Changes*

a. Preliminary Clearance Changes Changes which usher in those frankly designed to replace hyalinized segments of muscle begin early The necrotic material must be removed to make way for the ingrowth of sprouts from adjacent surviving muscle cells In the absence of complications such as infection or rupture of the muscle acute inflammatory cell infiltration is mild, and vascular dilatation and edema are not appreciable. The sarcolemmal tubes come to contain a variety of cells engaged upon phagocytosis and removal of hyalinized material The differentiation and characterization of these cells is very difficult and attempts to predicate the nature of each particular one in the crowd which fills the "Muskelschläuche" have failed to reach agreement The various aspects of this problem will be considered below (Section D) But whatever their source, the activity of these cells results in a clearing of necrotic material from the interior of the sarcolemmal tubes, making way for active regeneration. It seems that polymorphs and monocytes (from the blood) local histiocytes, and perhaps cells derived from the actual muscle fiber itself ("Muskelkörperchen") all play a part in the process. Some of these phagocytes die locally (Forbus 1926a) and products of their disintegration may then be carried away in the lymph stream (Pfuhl 1937) Clearance is rapid signs of new muscle fiber formation are apparent long before clearance is complete and the two processes go on side by side.

b New Muscle Fiber Formation Nuclear proliferation, noted above as a

very early change within affected segments of muscle fibers, appears to be primarily a response to irritation (Volkmann, 1893) Since these nuclei are important in regeneration, nuclear proliferation may be regarded as the first step in the process Forbus (1926a) attempted to distinguish between proliferated sarcolemmal nuclei and muscle nuclei embedded in the sarcoplasm independently of the sarcolemma, deriving "the greater part, if not all" of the new nuclei from the latter His criteria of distinction, however (appearance of nuclear chromatin presence of nucleoli, etc.) are not very firm and most observers would not depend upon them (cf Adams *et al.*, 1953) In either case, division appears to be amitotic. The first cells "pavement" the walls of partially emptied sarcolemmal tubes but later come to fill them. Around the new nuclei, basophilic cytoplasm accumulates in increasing amounts, apparently from the left over sarcoplasm of muscle fibers (Hoffmann, *1868*) Sometimes they become multinucleated (*"Muskelplatten"*) Intermingled with them may be obvious phagocytes which have not yet accomplished their scavenging task. Some of the newly formed muscle cells in the central part of the sarcolemmal tubes may undergo *degeneration apparently as a result of inadequate nutrition* (Hoffmann, 1868 Volkmann 1893 Forbus 1926a)

Much confusion has arisen in the literature from a failure to appreciate the rather different course which regeneration takes with varying degree of antecedent degeneration. The above description, for example, pertains to a localized degenerated segment of muscle fiber in which sarcolemma remains intact while the contained sarcoplasm and myofibrils undergo waxy degeneration. This is the common state of affairs in Zenker's degeneration, and under these conditions regeneration seems to take place from the "Muskelkörperchen" or muscle corpuscles which have been formed from proliferated sarcolemmal and muscle nuclei The process has been described in detail by Volkmann (1893) and Forbus (1926a) and is essentially a "discontinuous" one, in that individual muscle corpuscles increase in size become multinucleated and spindle-shaped, and then develop striations within their cytoplasm or it may be that several of the muscle corpuscles come together to form a plasmodial mass which then elongates and *differentiates in the same way* Regeneration is in both cases "discontinuous," taking place from discrete foci dotted along the degenerated segment. This corresponds to the "embryonic type" of regeneration of Durante (1902) Under such conditions, there is no need for

muscle sprout to grow into the degenerated segment from adjoining surviving muscle and sprouting into it is not observed.

When, however the initial insult to a muscle segment or to a whole fiber has been so great that the sarcolemma is completely devitalized over some extent, then no longer are surviving "seedlings" located along the defective stretch and regeneration must necessarily depend upon ingrowths into the dead segment from the surviving fibers at its margins. This is essentially the sort of regeneration which takes place after more severe experimental injuries such as crushing Naturally, both forms may be combined. The situation has been stated by Volkmann (1893) with startling clarity in view of the polemics indulged in by later authors, and deserves quotation in full (page 322) After describing the "discontinuous" and the "continuous" (sprouting) form of regeneration he goes on to point out that " both types of muscle regeneration present no incompatibility but are rather manifestations of a single principle. They may occur simultaneously In addition there are transitional forms between the two types, in that multinucleated muscular elements which arose by a discontinuous genesis may become continuous with the old muscle fibre stump as they become fibrillated, and may undergo their further evolution in association with it. On the other hand young muscle cells may bud off and develop further in a discontinuous fashion."

It so happens that Zenker's degeneration as seen in human material seldom involves destruction of the sarcolemma over any important extent, so that the "discontinuous" or "embryonal" type of regeneration from discrete foci along the course of the defect is common. Obviously a scaffolding of surviving sarcolemma is of great help in accomplishing reconstitution of muscle fibers. Should, however the sarcolemmal sheaths be destroyed then the muscle sprouts which grow in from the adjacent muscle fibers are directed by endomysial planes. It is because of the survival of sarcolemmal sheaths that regeneration in Zenker's degeneration is very good connective tissue being formed only when complications such as muscle rupture of hematoma have occurred.

C. Experimental Production of Zenker's Degeneration

It is clear that Zenker's waxy degeneration is not a condition specifically produced in humans under particular circumstances, but rather a particular stage in a sequence of changes which can be induced in muscle fibers by a variety of damaging influences. The

appearance of waxy change is actually a late phase, being preceded by granular and hyaline changes. Fishback and Fishback (1932a) summarize the sequence as follows (a) slight granular clouding with swelling and dimming of cross striations, (b) edema of fibers with prominent longitudinal fibrils, (c) vacuolation, (d) true granular degeneration which may be either albuminoid or fatty and (e) waxy degeneration with further lumpy disruption. They were able to produce waxy degeneration by a variety of chemical, bacterial, and pharmacological insults to striated muscle. Pfühl (1937) also produced the degeneration by subcutaneous injection of trypan blue in rabbits. *Minor differences in appearance of the waxy change seen in typhoid fever as compared with that in experimental lesions were described by* Thoma (1906 1909 1910) but do not seem to be of importance.

Attempts have been made to establish a common mechanism by which so many different noxious stimuli may produce the same change. Wells (1909) sought to relate it to lactic acid production locally but this has not been confirmed (Fishback and Fishback 1932b) and at the moment the question remains open.

D The Problem of the Cells inside the "Sarkolemmschläuche" of Waldeyer

"No changes in relation to the healing of wounds have been more difficult to interpret than those in muscle." (Dawson, 1909) The difficulties arise because of (a) the close association between degeneration and regeneration changes and (b) the similarities in appearance of elements derived from proliferated sarcolemmal nuclei, endomysium, and endothelium. Once the borders of the muscle cells have disappeared, it becomes very difficult to recognize the descent of individual cells.

The problem in fact dates back to the years immediately following Zenker's description of waxy change. Waldeyer (1865) who first emphasized the importance of regenerative changes in this condition and described the sarcolemmal tubes crammed with phagocytic cells as "Muskelzellenschläuche," was led to suggest this name because he thought that many cells were produced by amitotic multiplication of sarcolemmal nuclei producing "Muskelkörperchen" whose cytoplasm too was derived from surviving sarcoplasm. Thus he emphasized the production of cells within the sarcolemmal tube while Zenker had attached more importance to changes in the endomysium ("peri-

mysium internum") He also suggested that the "Muskelkörperchen," in addition to forming free cells might develop into a syncytium from which differentiation could occur in the direction of muscle fibers or connective tissue (Waldeyer 1865 pp 506 507) This speculation is interesting to compare with Levander's suggestion, discussed below that mesenchyme may be "induced" to form muscle fibers. Waldeyer's ideas were elaborated by several workers over the next few years. Thus Weber (1863 1867) maintained that cells derived from muscle cells could become transformed into "pus cells," contravening the idea of specificity of adult tissues then being canvassed. Since his time, many observers have agreed that Waldeyer's interpretation is reasonable but extremely difficult to prove correct or incorrect (Le Gros Clark, 1946) The opposite view that the phagocytic cells seen within Waldeyer's muscle cell tubes were not really formed there but were cells which had migrated in from outside, was maintained by Güssenbauer (1871)

In an attempt to distinguish the different sorts of phagocytic cells which are obviously at work within the sarcolemmal tubes, some workers have employed vital dyes. A remarkable early attempt to do so was made by Janowitsch Tschainski (1870) who injected aniline dyes intravenously at various stages during degeneration and regeneration in experimental wounding He noted the capacity of some cells to take up vital dyes but was unable to draw any firm conclusions from his experiments. Much later Kiyono (1914) confirmed that some of the cells in the sarcolemmal tubes would take up dye while others would not and he regarded these latter as of truly muscular origin. However he could not be sure that they too did not help in the phagocytosis of degenerate contents of the tubes.

Polymorphonuclear leucocytes and lymphocytes present within and around tubes are obviously of vascular origin, and are not usually numerous unless rupture of the muscle or infection has occurred. The function of the lymphocytes is no more clear here than elsewhere in the body and it is also difficult to see what local function may be ascribed to the plasma cells which may also be present in small numbers.

Most attention has been paid to the various types of phagocytic cell within the sarcolemmal tubes. Adams *et al* (1953) maintain that it is possible to distinguish phagocytic cells of muscular origin by their compact and rather heavy chromatin, together with the absence of a prominent nucleolus, but the distinction is difficult and unreliable

(Forbus, 1926a). The same author (Forbus, 1926b) used the method of vital staining to analyze the phagocytic cells which appeared after intramuscular injection of irritants such as alcohol, phenol, or boiling water. In order to distinguish pre-existing local histiocytes, which may wander into the sarcolemmal tubes subsequent to the damage from nonphagocytic cells, he stained up the experimental animals as a preliminary with trypan blue, thus labeling all histiocytes by the blue granules in their cytoplasm. After allowing an interval for the disappearance of the dye from the plasma, lesions were made. Under these conditions, he found that some of the phagocytic cells within the tubes were dye-labeled while others, morphologically identical with them, were not. Presumably the stained cells were histiocytes which had wandered in, while the unstained cells were produced after the damage, though the author adduces reasons for setting aside this conclusion. Thus in further experiments he showed that cells which were unquestionably of muscle origin could be induced to take up dye. It is recognized that the interpretation of seemingly simple vital dye injection experiments may be difficult and that cells which are obviously phagocytic may nevertheless fail to stain (Field, 1956). Pfühl (1937) produced degeneration by local injection of trypan blue and concluded that "Muskelkörperchen" derived from proliferated sarcolemmal nuclei and sarcoplasm may become converted into phagocytes. This idea is not new: reluctance to accept the possibility of transformation of such specialized adult tissue should perhaps be somewhat less since the work of Weiss (1944) on the *in vitro* transformation of cells of neural origin into macrophages. Moreover Chèvremont (1940, 1948) claims to have observed the formation of macrophages from muscle cells in tissue culture, and Betz (1951) has described the same change *in vivo* under conditions of ischemia. Mauer (1939), Altschul (1942), and Godman (1957) have also ascribed a less rigid specificity to muscle cells, claiming that cells derived from sarcolemmal nuclei and sarcoplasm may be converted into connective tissue cells, a possibility already suggested by von Opel (1901) and indeed by Waldeyer (1865). In lower animals, of course, such lability is well recognized (Thornton, 1938).

1. *Nuclei*

In addition to frankly phagocytic cells within the sarcolemmal tubes, many cells found therein are concerned with regeneration of

fibers. These cells are formed from proliferated sarcolemmal nuclei which have acquired a basophilic cytoplasm from remnants or the original sarcoplasm. They may be individual cells or may come to make up plasmodial plaques ("Muskelplatten") produced either by fusion of individual cells or failure of cytoplasm to divide after nuclear division Opinions differ as to whether proliferation of nuclei takes place by mitosis or by amitosis. Thus Volkmann (1893) appears to have observed amitosis of sarcolemmal nuclei in the early stages, followed later by mitotic divisions in the young muscle fibers. Le Gros Clark (1946) noted occasional mitotic figures in the sarcolemmal sheath cells of tubes occupied by regenerating fibers, but he could not be sure that many did not in fact belong to histiocytes still remaining within them. In common with the majority of previous workers, he ascribed the striking increase in number of nuclei to amitosis. After colchicine there were, for example, many arrested mitoses in connective tissue cells but none could be definitely assigned to regenerating muscle cells. Altschul (1947) made similar observations with colchicine and Godman (1955) found that the drug was without effect on the number or appearance of nuclei in rapidly growing muscle sprouts in tissue culture. More recently Godman (1957) has reported the finding of only one instance of a mitotic figure in a sarcoplastic ribbon either *in vivo* or *in vitro* Despite this, the increase in number of nuclei is enormous and in the absence of direct evidence, has been assumed to take place by amitosis. The grouping of nuclei in doublets or triplets, their arrangement in columns, and the frequent presence of indentations certainly support this view Lash *et al* (1957) believe that mitosis occurs only in the mononucleated cells which are prominent in the early stages of regeneration, and not in multinucleated masses within sarcolemmal tubes. They did not, indeed, find any convincing evidence of mitosis either and suggest that the accumulation of centrally placed nuclei is the result of mobilization rather than proliferation a conclusion they support by measurements of DNA content of "regenerating" nuclei. Konjetzny (1953) was of the opinion that the great number of nuclei was due rather to degeneration of sarcoplasm than to actual multiplication. It is thus apparent that the source of the very numerous nuclei seen in histological preparations of regenerating muscle is by no means clear All observers are agreed that mitotic figures are very rare and inadequate to account for anything like the number of muscle nuclei present. Hence, even though convincing

"fibres from one side of the wound appear to join, but it is granted that this may be an illusion as it is very difficult to follow the fibres in serial section." Jones (1949) showed that when the rectus muscle of the dog had been allowed to heal after clean division, stimulation of one part of the muscle led to contraction in the other portion beyond the scar. Gay and Hunt (1954) working with the rectus abdominus and tibialis anterior muscles of the rat, thought that direct union of muscle sprouts coming from the ends of the divided muscle fibers could take place. Indeed their "impression was that the majority of the transected fibres had reunited." More recently Cotte and Inglesakis (1956) removed segments of the esophagus of the dog (which is composed of striated muscle) and traced the process of healing. Despite the inevitable sepsis and the size of the gap they made, they found a surprisingly full regeneration of muscle fibers, beginning with multinucleated outgrowths from the divided fiber ends as early as the second day. They thought the buddings from either side met and fused, and that the rate of repair was of the same order as that estimated by Le Gros Clark (1946) in the rabbit. A curious finding was the persistence of muscle buddings even as late as 226 days, when some degenerated fibers, too, were still present.

That muscle may regenerate so completely has sometimes been claimed in the past by surgeons examining human cases at operation, and sometimes on the basis of animal experiments. Thus Bier (1917) observed complete restoration of muscle fibers after large excisions, but Martin (1919) and Bundschuh (1923) were unable to confirm this. More recently Horn and Sevitt (1951) found well marked regeneration in the tibialis anterior muscle after degeneration consequent upon rupture of the popliteal artery.

In the process of healing across a defect in muscle fibers, the greatest importance rests with the endomysial sheaths which act as directing planes for muscle sproutings in much the same way as to neurilemmal tubes in the process of regeneration of peripheral nerve. When injury is such that sarcolemmal tubes remain virtually intact, for example in Zenker's degeneration or in localized experimental freezing of muscle, regeneration is perfect. When, however, sarcolemmal tubes are destroyed, then the degree of reconstitution of muscle fibers depends on (among other things) the extent to which the more resistant endomysial connective tissue planes have survived. The pronounced influence of these was clearly shown by Le Gros Clark (1946), who found in ischemic

lesions that fibroblasts invade the tissue in advance of muscle sprouts and form new endomysial tubes to reinforce or reconstruct the old ones and so reproduce the original pattern of the muscle when the muscle sprouts follow them in. Moreover if a strip of muscle be excised and reimplanted after rotation through an angle of 90° then the ingrowing muscle sprouts may be seen to bend round and follow the new line of endomysial planes.

4 *Maturation of Myoblasts*

Individual myobrils usually appear coursing along the edge of a young muscle cell. They can make their appearence quite early and may be seen in isolated muscle cells which have not yet joined up in the making of a continuous fiber but usually they are not prominent at this stage (Adams *et al* 1953) Longitudinal striations appear before the transverse striation, and Forbus (1926a) indeed found the latter to appear very late. Le Gros Clark (1946) however found that transverse striations could be seen as early as the sixth day of growth and that they were very distinct after 2 or 3 weeks. Fishback and Fishback (1932) found them to be well advanced by the twelfth day Obviously great variations occur under different natural and experimental conditions and much depends upon the vitality and vigor of the regeneration process, so that generalization has limited interest for an individual case. It is, however clear that longitudinal striation precedes transverse and the general sequence of appearance of the latter follows that in embryogenesis the A discs appearing before the Z. The same sequence is seen in the regeneration of skeletal muscle after Coxsackie virus infection (Volume III Chapter IV Section VI E, 3)

5 *Time Scale of Changes*

Only a very general indication can be given of the time sequence of changes in regeneration. Von Meyenburg (1929) has summarized the main times given in the literature as follows

Nuclear proliferation takes place 4 to 6 hours after injury

Sarcolemmal tubes and giant cells appear on the 2nd to 5th day spindle-shaped muscle cells on the 3rd to 5th day budding of surviving muscle fibers on the 6th day onwards and lasting 6–8 weeks [though Cotte and Inglesakis (1936) found them much later (see Section III D 3)] young muscle fibers on the 6th to 8th day and cross striation during the course of the 2nd to 3rd week.

When cautery is used to make a larger defect, then the repair process is naturally not so perfect and depends upon the degree of tissue destruction. The first stage in regeneration is one of demarcation of necrotic tissue from surviving muscle fibers adjacent to the burn, a process usually taking about 5 days. Under the microscope, the dead fibers are structureless and hyalinized and practically all the nuclei are necrotic. Reparative changes take place at the margin of the necrotic mass and from the beginning of the second week, this region is characterized by the presence of muscle cells, giant nucleated plasmodial masses, and muscle buds, all mixed with proliferating connective tissue which invades the resorbed necrotic muscle. Young muscle fibers grow into the newly formed connective tissue from both sides. This "muscularization of the scar" lacks the guiding influence of endomysial planes and is irregular and far from complete. Indeed, even after weeks or possibly months the regeneration zone does not as a rule exceed 1 or 2 mm., the rest of the interval being filled with scar tissue.

If the heat applied is less severe, e.g. as in the experiments of Denny-Brown (1951) where a metal rod heated to 85° C. was placed across the surface of a muscle for 1 min. then a local coagulated band of fibers showing the Zenker type of waxy change is produced. Regeneration under such conditions is much more extensive.

C. Ischemia

The effects of ischemia upon muscle has been much studied both in the human and in the experimental animal. Extensive ischemia results in muscle death and replacement by connective tissue as in the well-known Volkmann's ischemic contracture. In general, muscle fibers are more susceptible to this form of injury than is connective tissue, so that after a more limited ischemia, connective tissue endomysial planes survive and as a result regeneration is well marked and effective. Even when ischemia has been quite severe in human cases, this may be so. Thus Horn and Sevitt (1951) followed regeneration in the *tibialis anterior* muscle after necrosis due to rupture of an aneurysm of the popliteal artery. They noted well-marked regeneration from surviving superficial muscle fibers, apparently by a process of budding similar to that described by Le Gros Clark, but they remarked on the presence of remnants of ischemic muscle buried within the newly formed tissue. Phagocytosis and removal of this necrotic material was very slow and some was found to be present even after 18 months. They thought this

persistence might be due to poor circulation for in Le Gros Clark and Blomfield's experiments (1945) revascularization had been rapid and was followed by reconstitutional changes.

The technique used by these latter authors of ligaturing off the main vessels to the anterior tibial muscles in rabbits affords an excellent method of studying regeneration in a muscle whose general architecture has been preserved just as in Zenker's degeneration of typhoid fever and the sequence of changes in both is similar The occurrence of conchoidal plates (fragmentation along Bowman's lines) noted by Le Gros Clark in his ischemic lesions, has been discussed above. Le Gros Clark and Wajda (1947) estimated regeneration of muscle fibers along sarcolemmal tubes to take place at about 1 0–1.5 mm. per day under the conditions of their experiments (Section III D 5)

Harman (1947) has studied the histological changes which take place in acute ischemia and drawn attention to the accentuation of cross striation in the early stages and its relation to fragmentation across the Z discs. Later (1948) he showed that even following brief periods of ischemia further extension of necrosis was not prevented when the vascular occlusion was released as the effective duration of blood deprivation is prolonged by stasis and capillary damage.

D Trauma

Fishback and Fishback (1932) have followed events in the gastrocnemius muscle of the rabbit when it is mildly contused by gentle blows from a light rubber-covered iron rod. They reported that evidences of regeneration in the form of muscle sprouts with multinucleated tips were to be seen as early as 24 hours after injury but that they were often less prominent at 72 hours. In explanation, they suggested that many of the earliest sprouts were sent out by muscle fibers which were in fact damaged and not able to maintain the reparative attempt. It seems probable however that many of the "sprouts" seen at 24 hours were really ruptured sarcoplasm with early nuclear proliferation Repair was well marked at 8–10 days, with clumps of newly growing fibers up to 1 mm. or more in length. They concluded that, if the sarcolemma is not too extensively destroyed and the stroma remains, then "these together with surviving muscle nuclei can form the integral factors for muscle restoration." Again, it is seen that emphasis is laid upon survival of sarcolemma and endomysial planes as features of great importance in regeneration.

E Injection of Chemical Substances

Kraske (1878) and Volkmann (1893) were among the earlier workers to use this method of inducing muscle degeneration for the study of subsequent repair Both used a few drops of concentrated carbolglycerin in rabbit muscle, and the same method was also employed in some experiments by Forbus (1926b) while Levander (1941) used an alcoholic bone extract in some of his experiments. Pfühl (1937) relied on the chemical irritation produced by trypan blue solution.

It is no more easy to follow repair changes in such experiments than after ischemia or other types of injury and indeed complicating factors seem to be introduced. Thus, Forbus (1926b) observed cartilage formation during the repair of his damaged muscle, once again emphasizing the lability of connective tissue. He described reproduction of new muscle cells as taking place by mitosis, but the difficulties of recognizing these young structures must be remembered. He thought that new muscle fibers were produced both by the "continuous" and the "discontinuous" processes. Another disadvantage of this method of studying muscle regeneration is the considerable amount of connective tissue which is as a rule formed for this may lead to much distortion which in turn may produce errors of interpretation. It is for this reason that Le Gros Clark (1946) felt unable to accept Levander's (1941) evidence of induction phenomena in the vicinity of his chemically-induced lesions. The attempts at regeneration may be marked but they are strangled by dense connective tissue overgrowth.

Muscle is not very responsive, by and large, to general chemical poisons. The outstanding exception is the substance "plasmocid [8-(3-diethylaminopropylamino)-6-methoxyquinoline dihydrochloride] Hicks (1950) who tested the toxicity of this compound from the point of view of its possible use as an antimalarial drug, found it to have a severely inimical effect upon skeletal and cardiac muscle. In the former dissolution of fibers occurred within 6–12 hours, accompanied by edema and a scant polymorph infiltration. Within 24 hours, histiocytes had appeared in large numbers and showed many mitoses. Proliferation of sublethally affected muscle nuclei was well marked apparently by amitosis although mitotic divisions were seen in a relatively well preserved fibers. After colchicine administration (Adams *et al* 1953) it was not, however certain that mitotic figures really ever belonged to

muscle nuclei. Regeneration was rapid and well marked evidently by muscle sproutings along intact sarcolemmal tubes and also by the "discontinuous" process, so that after 30 days or so reconstitution was practically complete—a result which is in accordance with expectation in view of the maintenance of the general architecture of the muscle and the existence of "seedling" foci along the course of the sarcolemmal tubes. Hicks found the masseter lingual, pharyngeal, and eye muscles to be most affected in his rats and Adams *et al* correlate the severity of lesions with activity of the muscles.

F Simple Transection

Just as in the case of peripheral nerve regeneration, a good deal depends upon how accurately the divided ends are opposed and the extent to which a hematoma develops between them. Accurate apposition is not always easy to secure (Dawson, 1909) and may account for some of the discrepancies in the literature. A good early account of the healing of transected muscle was given by Askanazy (1890) Millar (1934) and Adams *et al* (1953) have given substantially similar descriptions. In fetal muscle, events follow the same course, though they do not appear to be so markedly dependent upon endomysial pathways (Hess, 1954)

Nonviable muscle fibers in the immediate vicinity of the transection undergo hyaline degeneration and so do any fragments of fiber isolated along the knife track, while polymorphs and fibrin accumulate in it. Degenerated ends of surviving fibers become detached and are dealt with by phagocytosis, while from their healthy bared ends budding takes place in the usual way Millar (1934) emphasizes special appearances in the nucleoli of proliferated sarcolemmal cells, but Adams *et al* could not consistently find them Nuclear division appears to be amitotic and multinucleated muscle "giant cells" are found in the midst of the central necrotic material. These giant cells seem to contribute to the healing so that the new muscle cells are formed partly by a "continuous" and partly by a "discontinuous" process, maturation taking place in the usual way with cross striations appearing later than longitudinal. Under ideal conditions (when tissue destruction is minimal and there is little fiber retraction or hematoma formation) healing may be very good and leave little more than a slight local increase in connective tissue. Gay and Hunt (1954) investigated the problem of union of muscle wounds from the special standpoint of the actual grow

ing together of muscle fiber sprouts coming from opposite sides of the wound. They found the rate of growth of these sprouts to be rather less than the 1 0–1.5 mm. per day suggested by Le Gros Clark (1946) possibly because in the latter's experiments (ischemia) well preserved and continuous endomysial planes were available to the ingrowing fibers. The authors were of the opinion that a considerable union of muscle buds from the two sides did, in fact, take place (Section III D 3)

V EXPERIMENTS WITH HUMAN MUSCLE

A. Normal

Volkmann (1893) carried out intramuscular carbol-glycerin injection experiments after preliminary cocainization, in limbs which were doomed to amputation for some condition such as tumor growth. Cases were selected in which the muscle was otherwise normal and examination carried out from 6 hours to 43 days after injection. The *changes were essentially similar to those in his rabbit injection experiments*, although the injected material tended to spread more readily in the coarsely fasciculated human muscle, producing a very "banded" lesion.

Walton and Adams (1956) used a sterile suspension of carbon particles in a mixture of oil and alcohol as a means of producing a readily identifiable lesion in human muscle which was later removed at an operation undertaken for some other purpose. As did Volkmann, they found a close parallel between the sequence of changes in the rabbit and in the human the process being perhaps *a little less rapid in the* latter in the earlier stages, but reaching approximately the same stage by the 10th day They found both buds and isolated spindle cells to take part in the regeneration process.

B Pathological

Walton and Adams (1956) also examined the regenerative capacity of muscle in clinical cases of muscular dystrophy neuropathic atrophy and polymyositis, using volunteers who were willing to undergo muscle biopsy *They concluded that "impairment of the regenerative potential* of the muscle-cell is not a primary effect of either dystrophy or denervation. But a muscle fibre in an advanced stage of atrophy or degeneration, as a result of either denervation or muscular dystrophy loses its ability to regenerate."

VI. EFFECT OF DENERVATION AND MUSCLE TENSION UPON REGENERATION

Kirby (1892) using rabbits, investigated the effect of denervation upon subsequent regeneration in a damaged muscle. The calf muscles were ligated in their upper third until the lower part had assumed a dark blue appearance. Usually the ligature was *in situ* for about 3–3½ hours. It was then removed and the wound sown up In some cases preliminary division of the sciatic nerve was carried out. Histological investigation showed that, as an immediate result of the ischemia, muscle sarcoplasm was disrupted but connective tissue planes remained intact. On comparing the course of the very good regeneration which took place, Kirby was surprised to find that nerve section 5–10 days prior to the infliction of injury did not in any way hinder muscle regeneration. He also found that nerve section did not lead to muscle fiber degeneration for some weeks.

Denny Brown (1951) on the other hand, found that while the regenerative ability of muscle was unimpaired for the first 2 weeks after denervation, it was but feeble and abortive after 3 weeks. He pointed out that Kirby had limited his observations to the few days following nerve section. After 3 months, he found trauma to cause "first a proliferation of the nuclei and then fragmentation" (Adams *et al.*, 1953) Walton and Adams (1956) however found regenerative capacity to be retained for much greater periods in humans, recording active regeneration in an injured muscle which had been weak and atrophic for over 2 years and which had all the histological appearances of long standing degeneration. They concluded that it is only when denervation has produced profound atrophy of muscle cells that they fail to respond to injury Similarly Saunders and Sissons (1953) found recovery from a crush took place in exactly the same way in denervated as in intact muscle. They point out that because of the nature of the injury inflicted repair must in any case be taking place in many denervated muscle fibers even in an "intact" muscle. They extended the interval between nerve section and the injury to 3 weeks, using the rat as an experimental animal, and took precautions that reinnervation should not occur

The influence of tension in the muscle during regeneration following a burn has been examined by Denny Brown (1951) He showed that division of the tendon at the time of making the lesion, even though it prevented separation of the ends of the muscle fibers, slowed down

regeneration With an intact tendon, there was more distortion during the repair but the tension appeared to stimulate all phases of the process both actual formation of muscle fibers and their maturation.

VII EFFECT OF CORTISONE UPON MUSCLE REGENERATION

The effect of cortisone upon repair of rabbit skeletal muscle has recently been investigated by Simons and Hadfield (1953) and by Ellis (1955) The former used the crush technique of Le Gros Clark and found that 10–20 mg per kilogram body weight per day retarded the onset and rate of regeneration but did not alter its course or eventual outcome. In this respect, the effect of cortisone was much less pronounced than on bone repair Ellis found that healing was rapid once cortisone treatment was discontinued and that rabbits on potassium deficient diet showed changes similar to those found in the cortisone animals.

It seems possible that the delaying action of cortisone may be due to its depressant action upon mobility and phagocytic activities which are so important in the process of preliminary scavenging associated with muscle regeneration. Thus Paff and Stewart (1953) found cortisone to diminish ameboid activity of phagocytes in tissue culture. These factors have been discussed in another connection (Field, 1957)

VIII. REGENERATION OF CARDIAC MUSCLE

Many authors have examined the regenerative capacity of heart muscle and expressed varying opinions though the majority have found little (Fleischer and Loeb 1910 Christian *et al* 1911 Heller 1914 Karsner and Dwyer 1916 Collier 1922 Warthin, 1924 Bright and Beck, 1935 MacMahon 1937 Moritz and Atkins, 1938 Mallory *et al* 1939 King 1941 Harrison 1947 Walls, 1949 Ring, 1950) Just as in the case of skeletal muscle this variation is in some measure due to the different nature of the primary toxic influence after which regeneration has been studied. Thus some (e.g Harrison, Walls, Ring) deny that regeneration may take place at all, while others (Heller Warthin King for example) believe that it may

From what has been said above of the regeneration of skeletal muscle, it will be apparent that the character of the primary damage will be of importance in determining the vigor and success of any subsequent attempts at regeneration. Thus an insult which leaves the general architecture of the heart muscle undisturbed should present the best

chances for successful repair Such injury is best seen in the myocarditis which may accompany diphtheria and has been well described in detail by Heller (1914) and Warthin (1924) The essential lesion here is a toxic parenchymatous hyaline degeneration or necrosis associated frequently with fatty degeneration or with cloudy swelling Patchy waxy degeneration also occurs, and as in the case of the degeneration of skeletal muscle of typhoid fever the lesions are patchy in their distribution. It is followed by a reparative inflammatory process with foreign body giant cell formation as necrotic material is removed Warthin speaks of "perimysial tubes" becoming filled with detritus and inflammatory and regenerating cells in much the same way as occurs in skeletal muscle. Regeneration takes place later by an ingrowth of muscle sprouts into the tubes from adjacent surviving fibers. Bulbous swellings stuffed with myoblastic nuclei may be seen at the living ends of the muscle defects. Warthin was able to confirm Heller's (1914) findings in all respects and both support the view that heart muscle fibers do have a sarcolemma.

Associated with heart muscle fiber regeneration is some fibrosis and there is no doubt that this is a common result of most forms of cardiac injury just as it is in skeletal muscle injury Thus Martinotti (1888) noted only a slight proliferation of cardiac muscle nuclei (with mitotic figures) in the early stages after a stab wound of the rat heart, but did not regard this as of significance in healing which took place largely by fibrous tissue Anitschkow (1913) studying wounds of the rabbit heart found no true regeneration of muscle cells. He thought, however that damaged heart muscle cells could be converted through the loss of contractile substance first into "myocytes," with characteristic elongated nuclei showing serrated chromatin (Anitschkow cells) and then later into fibrocytes. Similar views with respect to skeletal muscle have been discussed above (Section III D)

Among more recent workers, Harrison (1947) concluded that rabbit cardiac muscle has not the same regenerative capacity as general somatic muscle and Walls (1949) too, failed to find evidence of regeneration after burning of the rabbit ventricular myocardium. However such thermal lesions are inevitably associated with some destruction of all the elements of the myocardium. Ring (1950) produced ischemic lesions in the cat and rabbit heart by ligation of branches of the left coronary artery He found revascularization of the infarct to take place within 14 days but no regeneration of muscle fibers. Thus he was in

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discussion of the structural and biochemical changes observed, represented by a rather extensive literature of the past 25 years, will naturally occupy a considerable segment of this chapter The remainder will deal with more limited information with respect to the effects of deficiency of certain water-soluble vitamins, choline, potassium, and tryptophan, the only other essential factors in nutrition shown to play a rôle in the metabolism of skeletal or cardiac muscle, or both, to the extent that in their absence structural alterations become apparent.

II. SKELETAL MUSCLE

A. Vitamin E Deficiency

Vitamin E, of which α tocopherol is the prototype, was first designated the antisterility vitamin however it has since come to be recognized as having an equally important function as an anti dystrophy vitamin. For the prevention of fetal resorption in the female rat, on the basis of which the vitamin was first discovered, it has been generally accepted that *α tocopherol as an intact molecule is required.* On the other hand, partial oxidation of the molecule to form the epoxide of α tocopherol destroys most of its antisterility properties without loss of antidystrophic activity whereas further oxidation to form the hydroquinone or quinone causes complete loss of antisterility activity without affecting its antidystrophic activity However α-tocopherylhydroquinone appears to have about the same antidystrophic activity as α tocopherol when administered intravenously to dystrophic rabbits (Mackenzie *et al.*, 1950) When tested by the oral route in vitamin E deficient hamsters, antidystrophic potency appears to be about one-third that of α tocopherol (West and Mason, 1955) In the rat, and probably also in other species, there is apparently no *in vivo* conversion of these oxidation products to α tocopherol (Mackenzie and Mackenzie, 1953)

Mackenzie (1953) in a review of this subject, suggests that vitamin E may be regarded as made up of two rather separate components α-tocopherol, the antisterility vitamin, and α tocopheryl hydroquinone, *the antidystrophy vitamin the former serving* also as a precursor of the latter The story may not prove to be as simple as this, for recent research (unpublished) in my laboratory carried out in collaboration with Dr S I Mauer and Dr G H. Rao indicates that α tocopheryl

hydroquinone administered orally does have antisterility activity, perhaps 1/10th to 1/20th that of α tocopherol in both male and female rats deficient in vitamin E

The latter phenomenon has been demonstrated in only a few animal species (rat, mouse, hamster guinea pig) whereas degeneration of skeletal muscles has been observed in all species of laboratory and domestic animals critically depleted of vitamin E. Consequently the interrelationships between vitamin E and muscle have become of real economic significance in the lifestock industry as well as matters of much interest to investigators concerned with the metabolic pathways in muscle.

As yet we have no clear understanding of the rôle played by vitamin E in maintaining the integrity of muscle. In a recent report presenting evidence implicating vitamin E as a co-factor in the cytochrome c reductase portion of both the DPN oxidase and succinate oxidase systems in the terminal respiratory chain in mammalian skeletal and cardiac muscle, Nason *et al* (1957) suggest that it also may function as part of a lipid sheath or as a cementing material maintaining cytochromes b and c in a spatial configuration promoting optimal reaction with each other Nason *et al.* also briefly review other postulations relative to vitamin F functions in metabolic process namely (1) as a component of the phosphorylating respiratory chain, perhaps as a link between respiration and oxidative phosphorylation (2) in some mechanism controlling oxidation that is unrelated to the levels of phosphate bond acceptor systems and (3) in purine metabolism and nucleic acid synthesis. It is apparent that there has been much recent progress directed toward the elucidation of the rôle of tocopherol in metabolic processes at the enzymatic level as well as to its secondary functions as an antioxidant. Further inquiries into the manner in which α-tocopherol and its oxidation products participate in these mecha nisms of action of vitamin E should also contribute much to a better understanding of the complex metabolic processes occurring within muscle cells.

Before discussing the biochemical and histopathological alterations of skeletal muscle in vitamin E deficiency a few general remarks may be pertinent First, it should be noted that the terms "muscular dystrophy nutritional muscular dystrophy" and "nutritional myodege neration" have been interchangeably applied to a state of muscular weakness, with or without obvious paralysis, associated with histologi

cally demonstrable lesions of the skeletal musculature, in animals deficient in vitamin E. These designations imply that the disorder represents a true myopathy primarily affecting the muscles rather than their nerve supply This is supported by the fact that the lesions differ from those seen in denervated muscles and by evidence that they are not preceded by any alterations in the peripheral nerves or their motor terminals. An extensive literature has appeared on this subject during the past quarter century The reader is referred to the early reviews by Evans (1940) and Pappenheimer (1943 1948) the more recent summary of Mackenzie (1953) on the experimentally induced dystrophy of laboratory animals, and the review of Blaxter and Brown (1952) dealing with observations on farm animals.

Secondly the lesions of skeletal muscle, which have been described for some twenty different species of laboratory and domestic animals, present a rather variable and confusing histopathological picture of muscle fibers manifesting no damage, intermingled with others showing sublethal injury progressive degeneration, and (usually) regenerative changes, since these fibers are seen in various profiles, depending upon the plane of section, there is provided a rather inadequate concept of events that may be occurring in different regions of any given fiber

Thirdly the character and intensity of the lesions are considerably influenced by many factors, such as (1) age of the animal and developmental maturity of the muscle fibers, (2) acuteness or chronicity of the deficiency state, (3) metabolic and structural differences between muscles in the same animal, and (4) species differences in response of the musculature to vitamin E deficiency These many influences naturally increase greatly the problem of comparing the lesions observed in different laboratory and farm animals.

1 *In Laboratory Mammals*

Lesions of skeletal muscles constitute a universal finding in all laboratory animals which have been subjected to vitamin E depletion. These include the rat, mouse, rabbit, guinea pig Syrian hamster Florida cotton rat, dog rhesus monkey chick, duck, and guppy fish (see review by Mason, 1954) Of these, the first five mentioned have received more extensive consideration than the others. Herbivorous species, as the rabbit and guinea pig are peculiarly susceptible and acute deficiency is often fatal. In other forms, the dystrophic state is

more slowly progressive and usually compatible with survival to late adult life.

Animals which have acquired appreciable stores of vitamin E through dietary sources retain it rather tenaciously so that rather long periods on deficient diet are required to induce manifestations of vitamin E deficiency For these reasons it has been customary in experimental studies to employ recently weaned animals, and even to place mother and suckling young on low E diets to further restrict tissue storage. This means that states of muscular dystrophy observed in most laboratory animals, and in farm animals also have been produced at periods comparable to infancy and adolescence, only in a limited number of species have chronic states of dystrophy in adult animals been studied.

a Symptomatology Evans and Burr (1928) called attention to an unusual type of paralysis occurring in the offspring of rats given just sufficient vitamin E to permit the successful completion of gestation and lactation. The young usually appear vigorous and healthy prior to the eighteenth day of life, but during the ensuing week there appears in all or in certain members of the litter a flaccid paralysis of the hind legs and often a flexor contraction of the fore limbs (Fig 1) Some affected animals become lethargic and succumb within a few days, perhaps because of extensive involvement of respiratory muscles others, in which the paresis is mild or moderate, show spontaneous recovery The affected animals and also many grossly unaffected members of the litter exhibit microscopically widespread lesions of the skeletal muscles which vary considerably in their intensity and in the extent to which individual muscles or portions of muscles are involved. When continued on low E diets the muscles of the young animals which recover spontaneously show rapid repair and little or no evidence of degenerative change until months later when the manifestations of chronic vitamin E deficiency appear

When lactating mice and their offspring are fed low E diets (with 18% lard and 2 % cod liver oil) which produce late-lactation paralysis in the rat, no symptoms appear even though muscle lesions are found histologically (Pappenheimer 1942 Tobin, 1950) However if the lard is replaced by increased amounts of cod liver oil, gross paralysis appears and the muscle lesions are much more severe and widespread (Tobin, 1950)

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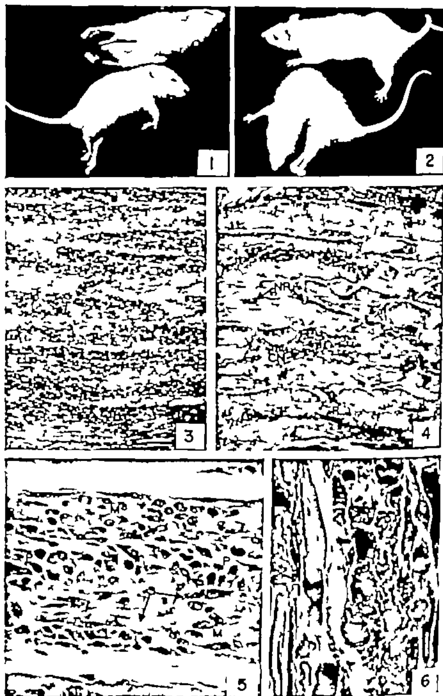


PLATE I

The onset of dystrophy in the rabbit, which often progresses rather rapidly has been divided into three stages by Mackenzie and Mc Collum (1940) There is an initial stage characterized by a twofold increase in the urinary output of creatine with no change in creatinine excretion usually followed by retarded growth and a decline in food intake. In the second stage, there is stiffness of the fore legs, some head retraction, slowness in righting when placed on the side, and accentuation of the earlier manifestations. In the final phase, the animals have great difficulty in regaining an erect position when prone or supine, show pronounced loss of body tonus when picked up and may become completely prostrate several days before death. Spontaneous recovery such as observed in weanling rats never occurs, but there is a remarkably rapid amelioration of all symptoms and manifestations of dystrophy if vitamin E therapy is instituted before these phenomena are too far advanced. In the guinea pig the course of events is much the same as in the rabbit.

In the Syrian hamster depletion of vitamin E from early life produces relatively little indication of muscle weakness or functional impairment until after 10 or 12 months of deficiency even though muscle lesions are evident histologically during the second month of deficiency and become progressively more severe as deficiency pro-

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FIG. 1 Late-lactation paralysis in 22-day-old rats, showing flaccid paralysis of hind limbs and flexor contraction of fore limbs.

FIG. 2 Paralysis of adult rats after chronic vitamin E deficiency Rats are litter mates 360 days old. In one rat (above) paralysis was arrested at the "spraddle" stage by vitamin E therapy (243th to 360th day) with only partial recovery from paresis. Other rat (lower) which received no therapy shows adductor contracture and atrophy of muscles of trunk and lower extremity From Mason and Emmel (1945)

FIG. 3 Skeletal muscle from young rat such as shown in Fig 1 Note extensive segmental necrosis of fibers separated by zones of edema and leucocytic infiltration. A few normal fibers are present (bottom) Magnification × 80. From Mason (1952)

FIG 4 Skeletal muscle from Syrian hamster 200 days deficient in vitamin E, showing segmental coagulation necrosis (CN) nuclear rowing (NR) and regenerative reactions (R) Magnification × 80 From Mason (1952)

FIG. 5 Enlarged view of two adjacent segments from Fig 3 transformed into densely cellular zones composed of macrophages (P) many muscle nuclei (M) and syncytial fusion of the latter to form basophilic, multinucleate, spindle-shaped strands (S) Magnification 360. From Mason (1952)

FIG. 6. Skeletal muscle of hamster showing extensive contraction-clot formation and varying degrees of necrosis of affected segments. Other fibers show internal nuclear rowing Magnification × 100. From West and Mason (1958)

gresses. There are certain resemblances to the rat subjected to chronic vitamin E deficiency except that for a comparable stage of depletion the microscopic lesions are much more extensive than in the rat (West and Mason, 1955)

The paralysis occurring in rats reared for prolonged periods (6 to 18 months) on low-E diets has been studied by many investigators and is described in detail in the monograph of Einarson and Ringsted (1938) Briefly there occurs an adductor weakness in the hind legs, leading to a spraddling or waddling type of gait (Fig 2) with slight incoordination as weakness and atrophy gradually extend to other muscles of the lower limbs, pelvic girdle, and trunk, the gait becomes more incoordinated and ataxic finally with loss of ability to walk, the animals drag the hind quarters along while supporting themselves weakly by the fore legs in moving about the cage, or lie most of the time on the side with hind legs contracted and spastic (Fig 2) Progressive hypesthesia and hypalgesia of the tails and lower extremities also occur Einarson and Ringsted (1938) and Einarson (1952) consider the clinical picture to be one of a neuropathy superimposed upon an earlier myopathy but others have regarded it as a true myopathy with some secondary sensory loss (see p 216) In early phases, the paresis is reversible with vitamin E therapy· however if spraddle of the hind limbs has persisted for some time, therapy does not result in full recovery (Fig 2) due probably to previously established abductor contractures. A similar paralysis has been observed by Menschik *et al.* (1949) in mice fed low E diets for 9 to 24 months.

b Histopathology In the early or acute type of dystrophy in suckling rats, as described by Pappenheimer (1939 1948) and others, the muscles are pale, moist, devoid of normal luster sometimes streaked, and gritty Microscopically there is widespread segmental necrosis of muscle fibers predominantly a contraction-clot type of reaction, and also an interstitial edema and leucocytic infiltration indicative of an inflammatory response (Fig 3) The rather short segments involved become swollen, lose their cross striations, and show transformation of the contractile substance into a somewhat homogeneous coagulum the latter soon undergoes dissolution as macrophages and leucocytes invade the sarcolemma sheaths. Muscle nuclei with their investment of sarcoplasm are released. They sometimes appear to fuse syncytially into narrow spindle-shaped or ribbonlike, multinucleate, basophilic strands at other times, they first become intermingled with macro-

phages and occasional leucocytes forming dense cellular cords within the endomysial framework of the segment (Fig 5) It is assumed, but difficult to prove that the myoblasts undergo some mitotic division. In the cellular cords, they likewise tend to fuse more or less longitudinally so as to give rise to the same type of multinucleate strand referred to above. In the latter myofibrils soon appear in increasing numbers between the sarcolemma and the large nuclei which occupy much of the interior of the developing fiber These fibers become more acidophilic through accumulation of myoglobin, increase in length and diameter acquire more and more myofibrils, and effect a peripheral positioning of their nuclei. There are thus formed new fibers to replace, in part at least, those previously lost through degenerative changes. This regenerative response of immature, youthful muscle, occurring as it does in the face of continued deficiency of vitamin E, is a truly remarkable phenomenon. It is of significance that paralysis rarely occurs before the eighteenth or after the twenty fifth day of life and that vitamin E therapy prevents the lesions if given as late as the seventeenth day but has doubtful benefit once the lesions are well established. This suggests an especially acute need for vitamin E during a particular period of biochemical and structural maturation and growth of the skeletal musculature. If vitamin E deficiency is continued after spontaneous recovery several months may elapse before lesions again make their appearance as will be indicated later these lesions are of a rather different character

Investigators have been perplexed as to why in certain muscles, great numbers of muscle fibers are damaged while in adjacent muscles, separated by only a thin fascial plane, there is little or no involvement or why more deeply situated fibers show extensive injury while more superficially located fibers may remain normal. Pappenheimer suggests that the better preserved fibers may have a richer blood supply or may be more immobilized by fascial attachments and thus less actively involved in muscular activities. In the latter connection, Pappenheimer (1940) has shown that immobilization of the gastrocnemius by section of its tendon or its nerve supply prior to the eighteenth day prevents the occurrence of muscle lesions.

Muscle lesions in suckling mice though less severe than in young rats unless intensified by an increase in dietary unsaturated fatty acids, are histologically quite similar to those described for the rat (Pappenheimer 1942 Tobin 1950)

In the guinea pig and rabbit, reared from early age on vitamin E deficient diets, there occurs a somewhat less explosive type of reaction in skeletal muscles than in the suckling rat, but the cellular reactions are much the same. There is, however a greater tendency toward replacement of injured fibers by adipose and fibrous tissue, indicating perhaps that the considerable regeneration of new fibers observed may fail to fully replace those lost through degeneration. Vitamin E therapy effects rather rapid repair structurally and biochemically By proper regulation of vitamin E intake, it is possible to induce alternate states of dystrophy and recovery for long periods of time, or to maintain a state of chronic dystrophy compatible with good growth and vigor (Mackenzie, 1942) In states of chronic dystrophy the lesions are characterized by a predominance of healthy muscle fibers between which are interspersed fibers showing degenerative and regenerative changes of a less acute type, resembling those of chronic deficiency in the rat.

Muscle lesions observed in the Syrian hamster reared on low E diets, in terms of rate of onset and intensity of reaction, are somewhat intermediate between the acute types seen in the suckling rat, guinea pig and rabbit and those observed after chronic vitamin E deficiency in the adult rat. They are particularly suitable for study of certain cellular reactions of dystrophic muscle. Furthermore, the hamster offers a particular advantage in that stained, fixed spreads of the cheek pouch permit study of individual fibers *in toto* for relatively long distances. This provides much more information regarding the extent to which segments of fibers are involved in histopathological changes and the variability in the nature of these changes than is possible through the study of histologic sections of muscle alone. The following description is based upon the observations of West and Mason (1955, 1958)

In the muscle lesions, it is possible to recognize, in addition to extensive nuclear rowing discussed later four rather distinct types of irreversible injury in fiber segments (1) a typical coagulative necrosis (Figs. 4 and 7) representing the most common type (2) formation of contraction clots, and necrosis of affected segments (Fig 6) (3) focal granularity and breakdown of myofibrils, with conversion of the segment into a highly granular mass (Figs. 15 and 16) (4) enlargement and dissolution of nuclei, associated with vacuolar degeneration (Figs. 17 and 18) It should be stated that the terms "hyaline" and

"Zenker's" necrosis, or degeneration, which have often been used in reference to both coagulation necrosis and contraction-clot necrosis, are considered undesirable and misleading because of their varied meanings and implications Contraction-clotting often results from immersion of excitable muscle in fixatives, but it also occurs *in vivo* in fact, it is the major type of reaction in late lactation paralysis in the rat and is also common in the rabbit and guinea-pig but is less frequent in the hamster and adult rat. The fate of such damaged segments is essentially as described for those involved in coagulation necrosis the same is probably true of segments showing focal degeneration of myofibrils and granular degeneration.

Coagulation necrosis is characterized by a rather rapid sequence of events involving proliferation (presumably amitotic) and irregular disposition of muscle nuclei within the fiber segment, loss of striations, and transformation of the cytoplasm into a somewhat granular coagulum (Fig 7) macrophages, accompanied by a few leucocytes, invade the segment and rapidly phagocytize the coagulum, so that there is left a highly cellular mass (the *Muskelzellenschläuche* of Waldeyer) composed chiefly of macrophages and muscle nuclei with their investing sarcoplasm, which may be called "myoblasts" (Figs. 8 9 and 10) The external form of the segment is preserved, and the endomysium shows little or no evidence of injury

Regeneration of damaged segments occurs to some extent through plasmodial sprouts or outgrowths from intact portions of the fiber adjacent to the zone of injury (Figs. 9 and 10) and to a greater extent through a process similar to that described previously for the suckling rat. In the densely cellular areas occurring within the endomysial framework of the injured segment (Figs. 8 and 9) myoblasts increase in number become spindle-shaped and sometimes multinucleate, and acquire a pronounced basophilia attributable to ribonucleic acid and therefore suggestive of active protein synthesis they soon become aligned parallel with the long axis of the segment and establish protoplasmic continuity with each other so that a true syncytium is formed (Figs. 5 11 and 12) this, in turn, becomes confluent with zones of regeneration arising through plasmodial outgrowths from intact portions of the fiber (Figs. 9 and 10) These events are seen especially well in intact fibers of the cheek pouch where injured segments may show different phases of the regenerative process over a distance of several millimeters. Later phases of the process, which presumably

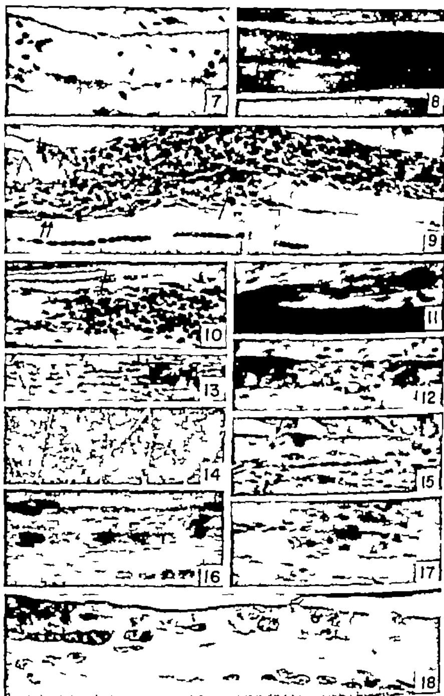


PLATE II

involve formation of myofibrils and allignment of the nuclei in internal rows with their subsequent disposition to a subsarcolemmic location, perhaps through mechanical forces exerted by the myofibrils, are

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All figures are photomicrographs of skeletal muscle of vitamin E deficient Syrian hamsters.

FIG 7 Typical coagulation necrosis as seen prior to invasion of mononuclear cells. Note irregular disposition of many muscle nuclei. Magnification × 200

FIG. 8 Area of dense cellularity (*Muskelzellenschlauche* of Waldeyer) continuous with normal segment of same fiber (at arrow) Nuclear rowing in other fibers. Magnification × 100. From West and Mason (1958)

FIG. 9 Area similar to that of Fig 8, in two adjacent fibers. Myoblasts show early regenerative response in subsarcolemmic region (double arrows) and as plasmodial outgrowths from intact portion of fiber (angle arrow) Internal nuclear rowing in other fiber Magnification × 200.

FIG. 10 Similar to Figs. 8 and 9 showing plasmodial syncytium (arrow) at junction of *Muskelzellenschlauche* with normal segment of fiber Magnification: × 200. From West and Mason (1958)

FIG. 11 Elongated, spindle-shaped myoblasts in zone of early regeneration. Cell in mitosis cannot be identified. This figure, and the following two figures, are from unsectioned fibers observed in spreads of the cheek pouch Magnification × 200 From West and Mason (1958)

FIG. 12 Later phase of regeneration, in which elongated myoblasts form a syncytium within the endomysial framework of the injured segment. Magnification: × 200. From West and Mason (1958)

FIG. 13 Unsectioned fiber with numerous nuclear rows of variable length but with preservation of normal striations; other nuclei are in subsarcolemmic position. Magnification × 100 From West and Mason (1958)

FIG. 14 Section from muscle of hamster given 10 days of vitamin E therapy after development of lesions similar to those seen in Fig 4 Necrosis has been arrested and eliminated, leaving only a few young regenerating basophilic fibers and many in which internal nuclear rowing (sublethal but not irreversible injury) had occurred prior to therapy and still persists. Magnification × 55 From West and Mason (1955)

FIG. 15. Segment showing focal breakdown of myofibrils and resulting granularity Several enlarged and irregularly disposed nuclei lie near the junction (arrow) with the more normal segment of the fiber Magnification × 200 From West and Mason (1958)

FIG 16. Portion of a segment similar to that in Fig 15 showing characteristic linear rowing of coarse granules. Magnification × 430. From West and Mason (1958

FIG 17 Segment showing enlargement and dissolution of irregularly disposed nuclei, with resultant vacuolation, yet with preservation of striations peripheral to the affected zone and in more normal portions of the fiber Magnification × 200. From West and Mason (1958)

FIG. 18. A similar segment showing a more advanced phase of the same process (upper right) and an earlier phase (upper left) in which large vesicular nuclei are irregularly distributed or arranged in short rows. Magnification × 430. From West and Mason (1958)

difficult to identify with certainty since they so closely resemble phases of another type of reaction designated "internal nuclear rowing," which is a particularly widespread phenomenon in muscles of the dystrophic hamster.

The latter reaction is seemingly initiated by proliferation and random distribution of nuclei within the fiber segment, with subsequent alignment of these nuclei (Figs. 4–6, 8, 9–13 and 14) in single or multiple rows of variable length in otherwise normal fibers. There is often a definite basophilia of the cytoplasm, especially in the internuclear zones. This is interpreted as a sublethal type of injury from which recovery can occur and fibers may remain in this state for prolonged periods without significant impairment of functions.

Focal breakdown of myofibrils with conversion of the affected segment into a granular mass (Figs. 15 and 16) is a slowly progressive change which may represent an irreversible type of injury occurring in fibers which for considerable periods of time have shown only nuclear rowing. The other type of injury mentioned is characterized by focal dissolution of enlarged nuclei and consequent vacuolar formation (Figs. 17 and 18) often with good preservation of striations in adjacent areas (Fig. 17). This condition may represent a more chronic and gradual alteration affecting segments in which randomly distributed nuclei fail to become arranged in rows, or segments in which nuclear rowing cannot be maintained. It is difficult to determine at just what point this gradual alteration of the segment reaches a state of irreversibility. Regeneration occurs primarily through proliferative activities of persisting hypolemmal nuclei in much the same manner as after other types of irreversible injury.

The phenomenon of nuclear rowing has usually been interpreted as a regenerative response. There is now good reason to believe that it represents, for the most part, a sublethal reaction to injury. Counts of nuclei of intact fibers in the cheek pouch indicate that the number of nuclei present in a given length of "rowed" fibers is about 50% greater than in normal fibers of comparable size and state of contraction; since mitoses have not been observed, it is assumed that an appreciable amount of amitotic division occurs. The similarity of such fibers to immature muscle fibers suggests that internal nuclear rowing may represent a process of dedifferentiation associated with increased resistance to sublethal injury from which recovery may occur when metabolic conditions become more favorable.

Following vitamin E therapy which promptly prevents further coagulation necrosis and other types of irreversible injury, there is a gradual reduction in the number of rowed fibers but some may be observed after several months of therapy The reader is referred to the report of West and Mason (1955) for details and illustrations of these changes. Apparently restoration of normal morphology in these fibers or in those representing true regeneration which may closely resemble each other at certain stages, is not always a rapid or simple process

Muscle lesions of chronic vitamin E deficiency in adult rats, compared to those in the hamster are much less extensive after comparable periods of deficiency internal nuclear rowing is relatively infrequent and the rows are relatively short. This suggests a significant species difference in range of reaction of muscle to mild injury During the first few months, coagulation necrosis and associated regenerative reactions involve segments of somewhat scattered fibers. Less frequently seen are segments in which nuclei show proliferation, vesicular enlargement, irregular distribution or arrangement in small clusters or short chains, and progressive dissolution with formation of irregular vacuolar spaces in association with granular breakdown of myofibrils. This vacuolar type of degeneration is basically the same as that described for the hamster

During subsequent months, the number of segments involved in both types of reactions increases gradually a few may show contraction-clot formation and necrosis. As deficiency progresses to more advanced ages (10 to 18 months) the incidence of coagulation necrosis appears to diminish in intensity while the vacuolar type of degeneration seems to involve increasingly larger numbers of segments until it becomes the predominant type of reaction present (Figs. 19 and 20) Repair processes are also in evidence at the site of breakdown of muscle fibers. In advanced dystrophy the large proportion of fibers showing injury especially that of the vacuolar type, often seems excessive in terms of the gross evidence of functional impairment observed in the animal. This gives rise to impressions that the vacuolar type of injury not only progresses very slowly compared to the other types of irreversible change but that some degree of functional activity may exist despite considerable nuclear disorganization and dissolution and appreciable loss of myofibrillar structure. There is need for careful study of the response of such fibers at short intervals after vitamin E therapy in order to better appreciate how much cytological alteration

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During subsequent months, the number of segments involved in both types of reactions increases gradually a few may show contraction-clot formation and necrosis. As deficiency progresses to more advanced ages (10 to 18 months) the incidence of coagulation necrosis appears to diminish in intensity while the vacuolar type of degeneration seems to involve increasingly larger numbers of segments until it becomes the predominant type of reaction present (Figs. 19 and 20) Repair processes are also in evidence at the site of breakdown of muscle fibers. In advanced dystrophy the large proportion of fibers showing injury especially that of the vacuolar type, often seems excessive in terms of the gross evidence of functional impairment observed in the animal This gives rise to impressions that the vacuolar type of injury not only progresses very slowly compared to the other types of irreversible change, but that some degree of functional activity may exist despite considerable nuclear disorganization and dissolution and appreciable loss of myofibrillar structure. There is need for careful study of the response of such fibers at short intervals after vitamin E therapy in order to better appreciate how much cytological alteration

difficult to identify with certainty since they so closely resemble phases of another type of reaction designated "internal nuclear rowing," which is a particularly widespread phenomenon in muscles of the dystrophic hamster

The latter reaction is seemingly initiated by proliferation and random distribution of nuclei within the fiber segment, with subsequent alignment of these nuclei (Figs. 4 6 8, 9 13 and 14) in single or multiple rows of variable length in otherwise normal fibers. There is often a definite basophilia of the cytoplasm especially in the inter nuclear zones. This is interpreted as a sublethal type of injury from which recovery can occur and fibers may remain in this state for prolonged periods without significant impairment of functions

Focal breakdown of myofibrils with conversion of the affected segment into a granular mass (Figs. 15 and 16) is a slowly progressive change which may represent an irreversible type of injury occurring in fibers which for considerable periods of time have shown only nuclear rowing The other type of injury mentioned is characterized by focal dissolution of enlarged nuclei and consequent vacuolar formation (Figs 17 and 18) often with good preservation of striations in adjacent areas (Fig 17) This condition may represent a more chronic and gradual alteration affecting segments in which randomly distributed nuclei fail to become arranged in rows, or segments in which nuclear rowing cannot be maintained. It is difficult to determine at just what point this gradual alteration of the segment reaches a state of irreversibility Regeneration occurs primarily through proliferative activities of persisting hypolemmal nuclei, in much the same manner as after other types of irreversible injury

The phenomenon of nuclear rowing has usually been interpreted as a regenerative response. There is now good reason to believe that it represents, for the most part, a sublethal reaction to injury Counts of nuclei of intact fibers in the cheek pouch indicate that the number of nuclei present in a given length of "rowed" fibers is about 50% greater than in normal fibers of comparable size and state of contraction since mitoses have not been observed, it is assumed that an appreciable amount of amitotic division occurs The similarity of such fibers to immature muscle fibers suggests that internal nuclear rowing may represent a *process of dedifferentiation* associated with increased resistance to sublethal injury from which recovery may occur when metabolic conditions become more favorable.

may occur before a critical threshold of irreversible injury is reached.

Vitamin E deficiency in other laboratory mammals such as the mouse, dog and monkey results in a histopathological picture essentially similar to that of the rat and hamster differing only in the relative incidence and intensity of the four major types of irreversible injury

At this point a few general comments are in order In a desire to simplify the histopathological picture as much as possible, there has been omission of certain points which should be given brief consideration. First, the lesions described are associated with little or no inflammatory reaction, with no significant alteration of vascularity and, except in the case of the rabbit and guinea pig, with relatively little replacement of muscle tissue by fibrous or adipose tissue. However there is evidence (Mackenzie, 1953) that degenerative processes can be appreciably accelerated in the rat by simultaneous deficiency in protein or in pyridoxine. Second, the lesions are significantly acceler

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FIG. 19 Longitudinal section of intercostal muscle of rat, after chronic vitamin E deficiency of 520 days, showing extensive vacuolar degeneration in fibers above and below a normal-appearing fiber Magnification × 335.

FIG. 20. Cross section of same muscle, showing irregular shape and distribution of vacuolar spaces and of nuclei in fibers possessing a reduced complement of myofibrils. Magnification × 335.

FIG. 21 Intercostal muscle of vitamin C deficient guinea-pig showing early type of degenerative change, involving coagulation necrosis (left) and reactions suggestive of regeneration (upper right) From Dalldorf (1929)

FIG. 22. Rabbit deprived of choline for 200 days, showing pronounced plasticity of the hind legs. From Hove and Copeland (1954)

FIG. 23. Muscle from choline deficient rabbit, showing numerous degenerating fibers and some increase in fibrous connective tissue. Magnification × 120. From Hove and Copeland (1954)

FIG. 24 Skeletal muscle of guinea-pig fed diet deficient in the anti-stiffness factor showing much necrosis and macrophagic invasion of muscle fibers. Magnification 110. From Harris and Wulzen (1950)

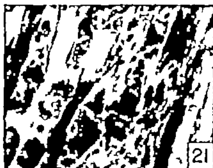
FIG. 25. Portion of ventricular myocardium, showing areas of fibrotic replacement of cardiac muscle following prolonged vitamin E deficiency in the rat. Magnification 35 From Mason and Emmel (1945

FIG. 26. Portion of uterine myometrium of rat after prolonged vitamin E deficiency showing extensive accumulation of brown, acid-fast, pigment granules in smooth muscle cells and in clusters of macrophages between circular and longitudinal muscle layers. Magnification 733 From Mason and Emmel (1945)

FIG. 27 Similar accumulation of pigment granules within smooth muscle cells in the wall of small artery (above) and vein (below) of vitamin E deficient monkey Magnification 230. From Mason and Telford (1947)

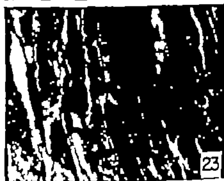












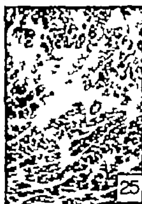






PLATE III

may occur before a critical threshold of irreversible injury is reached.

Vitamin E deficiency in other laboratory mammals, such as the mouse, dog and monkey results in a histopathological picture essentially similar to that of the rat and hamster differing only in the relative incidence and intensity of the four major types of irreversible injury.

At this point a few general comments are in order. In a desire to simplify the histopathological picture as much as possible, there has been omission of certain points which should be given brief consideration. First, the lesions described are associated with little or no inflammatory reaction, with no significant alteration of vascularity and, except in the case of the rabbit and guinea pig with relatively little replacement of muscle tissue by fibrous or adipose tissue. However, there is evidence (Mackenzie, 1953) that degenerative processes can be appreciably accelerated in the rat by simultaneous deficiency of protein or in pyridoxine. Second, the lesions are significantly accele

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FIG. 19. Longitudinal section of intercostal muscle of rat, after chronic vitamin deficiency of 220 days, showing extensive vacuolar degeneration in fibers above and below a normal-appearing fiber. Magnification: × 335.

FIG. 20. Cross section of same muscle, showing irregular shape and distribution of vacuolar spaces and of nuclei in fibers possessing a reduced complement of myofibrils. Magnification × 335.

FIG. 21. Intercostal muscle of vitamin C deficient guinea-pig showing early type of degenerative change, involving coagulation necrosis (left) and reactions suggestive of regeneration (upper right). From Dalldorf (1929).

FIG. 22. Rabbit deprived of choline for 200 days, showing pronounced plastic... of the hind legs. From Hove and Copeland (1954).

FIG. 23. Muscle from choline deficient rabbit, showing numerous degenerating fibers and some increase in fibrous connective tissue. Magnification 120. From Hove and Copeland (1954).

FIG. 24. Skeletal muscle of guinea-pig fed diet deficient in the anti-stiffness factor showing much necrosis and macrophagic invasion of muscle fibers. Magnification 110. From Harris and Wulzen (1950).

FIG. 25. Portion of ventricular myocardium, showing areas of fibrotic replacement of cardiac muscle following prolonged vitamin E deficiency in the rat. Magnification 35. From Mason and Emmel (1945).

FIG. 26. Portion of uterine myometrium of rat after prolonged vitamin E deficiency showing extensive accumulation of brown, acid-fast, pigment granules in smooth muscle cells and in clusters of macrophages between circular and longitudinal muscle layers. Magnification 235. From Mason and Emmel (1945).

FIG. 27. Similar accumulation of pigment granules within smooth muscle cells of the wall of small artery (above) and vein (below) of vitamin E deficient monkey. Magnification 250. From Mason and Telford (1947).

ated and intensified in all species, and especially in the rabbit and guinea-pig by increase in dietary unsaturated fats. It is generally assumed that tissue peroxides so formed may not directly inactivate vitamin E in the muscle cell but either interfere with oxidative or other enzymatic functions of the cell or produce serious alterations in the cell membrane or other structural component of the cell. *Third*, there occurs in most species studied a progressive accumulation of an inert, acid-fast pigment, particularly in segments undergoing the vacuolar type of degeneration and in macrophages of the adjacent connective tissues. This has been studied extensively in the rat (Mason and Emmel 1945) The pigment, which is either a lipoprotein or polymerized product of unsaturated fatty acids, occurs when vitamin E, functioning as an intracellular antioxidant, is insufficient to protect or stabilize unsaturated lipids of the cell. Since muscle lesions of lesser severity occur in the absence of dietary fat, and prior to the appearance of pigment when diets are high in unsaturated fats, the presence of the pigment is regarded as a secondary phenomenon which probably has no direct bearing upon the genesis of the lesions.

c Myopathy versus Neuropathy The muscle lesions of vitamin E deficiency in the various species studied have been universally regarded as purely myogenic in nature, except for conflicting opinions concerning the lesions associated with chronic vitamin E deficiency in adult rats. Certain investigators (Einarson and Ringsted 1938, Einarson, 1952, 1953 Monnier 1941) consider that the initial lesions are myogenic, but that with progressive deficiency there is superimposed a neurogenic type of lesion characterized by demyelinization of axons and gliosis in posterior spinal fasciculi and dorsal nerve roots, increasing accumulation of acid fast pigment in anterior horn cells with simultaneous diminution in Nissl substance and, eventually irreparable atrophy and sclerosis of sufficient numbers of these cells to superimpose a denervation type of atrophy upon the earlier myopathic lesions. The demyelinization and gliosis in nerve roots and posterior columns, which have been confirmed by others (Luttrell and Mason, 1949 Malamud *et al.*, 1949) probably explain increasing degrees of hyperkinesia and hyperalgesia which occur during advanced stages of the disorder A restudy of this problem in my laboratory by Mr Charles Willis has verified observations relative to extensive accumulation of acid-fast pigment in ventral horn cells, and in glial cells and macro-

phages elsewhere in the nervous system but has revealed only occasional sclerotic motor cells in the spinal cord Since these have been seen as often in the vitamin E supplemented as in the vitamin E deficient rats, they may be considered as phenomena related to old age rather than to the muscle lesions *per se* Furthermore, the evidence presented previously (p 213) indicating that the histopathological events occurring in the segmental injury of muscle fibers in chronic deficiency of adult rats are basically the same as seen in the hamster and other species, differing chiefly in respect to their more gradual evolution, seems not to justify the invoking of neuropathic mechanisms for explaining the muscle lesions observed. In other species, examination of the central nervous system has revealed no lesions due to vitamin E deficiency

Considerable attention has also been given to peripheral nerves and motor end-plates in various species studied. Motor end plates are reported to be unaffected (Rogers *et al.*, 1931) Pappenheimer 1939) or to be reduced in severely dystrophic muscle, followed by return to normal upon recovery (Telford, 1941) An atrophy of muscle spindles has been reported (Einarson and Ringsted 1938) which may also represent a secondary effect. Relatively little attention has been given to the electrophysiology of muscle after vitamin E deficiency Strength-duration relationships have been studied by Victor (1934) who reports considerable increase in rheobase and a moderate increase in chronaxie in the rabbit, and a parallel increase in both reactions in the skeletal muscle of dystrophic ducklings

In the preceding section an effort has been made to present in some detail the varied types of cellular reactions observed in skeletal muscle as they occur in different species of laboratory mammals at different ages and under different intensities of vitamin E depletion. The descriptions given reflect personal impressions of the author based upon histological study of the lesions in different species over many years, and upon descriptions of the lesions by many other investigators whose separate contributions comprise a very extensive literature There are obviously many imperfections in any attempt to detail specific cellular alterations and to relate them to any sequence or sequences of events which are presumed to occur within muscle fibers especially when the evidence is based upon the static morphology presented by fixed and stained sections. It is hoped that the descriptions and interpretations made will despite their shortcomings, serve

to emphasize the many gaps that exist in our knowledge of the histopathology of muscle and encourage others to contribute toward a more adequate understanding of the sequential events and intracellular mechanisms involved.

d. Biochemical Changes Much of our information on chemical changes in muscle after vitamin E deficiency is based upon the dystrophic rabbit and represented by the pioneer studies of Goettsch and Brown (1932) Victor (1934) Morgulis and Spencer (1936) Fenn and Goettsch (1937) Morgulis *et al.* (1938) Mackenzie and McCollum (1940 1941) Friedman and Mattill (1941) Houchin and Mattill (1942) and Kaunitz and Pappenheimer (1943) The subject has also been reviewed by Mackenzie (1953) It may be appropriate, however at this point to present a brief résumé of earlier and more recent studies concerning the more important constituents of dystrophic muscle.

Increased oxygen uptake of skeletal muscle after vitamin E deficiency first demonstrated in rabbits (Victor 1934) occurs also in the suckling rat, adult rat, hamster and chick (Friedman and Mattill, 1941 Houchin and Mattill, 1942 Kaunitz and Pappenheimer 1943), but, for unknown reasons, is not demonstrable in ducklings (Victor, 1934) which show much more pronounced muscle lesions than do chicks. The respiratory quotient and rate of glycolysis of muscle remains normal. Increased oxygen uptake may precede the appearance of histological lesions in young rats (Kaunitz and Pappenheimer 1943) and rabbits (Hummel and Melville, 1951) and is restored to normal in rabbits within as little as 4 hours after intravenous, and 10 hours after oral administration of α tocopherol (as shown by comparison of muscle obtained by biopsy and necropsy from the same animal) even though lowered creatine and increased chloride content of the muscle is not improved (Houchin and Mattill, 1942) These observations suggest that the increased O_2 uptake of dystrophic muscle, which may reflect functions of tocopherol as an intracellular antioxidant, represents a primary response to vitamin E deficiency whereas the other biochemical changes are secondary to this or to some other metabolic disturbance Efforts to study the effects of tocopherol on muscle slices *in vitro* have been hampered by difficulties inherent in getting a fat-soluble substance into an intracellular environment.

It has long been recognized that late lactation" paralysis in suck

ling rats usually appears between the seventeenth and twenty fifth days of life, and that vitamin E therapy given after the fifteenth to seventeenth days is usually ineffective in preventing the syndrome. It is also known that the major protein components of skeletal muscle (insoluble, actomyosin and soluble fractions) increase in concentration at different rates up to about the fifteenth day after which that of the insoluble fraction (scleroproteins) gradually decreases and that of actomyosin progressively increases (Herrmann and Nicholas, 1948) It is therefore of interest that in members of litters showing late-lactation paralysis there occurs a distinct reversal of the normal concentration of these two fractions between the eighteenth and twenty fifth days of life, and that in the course of spontaneous recovery (without additional vitamin E) there is a rather rapid restoration of this disturbed relationship (Rumery *et al.*, 1955) Furthermore, a similar imbalance between actomyosin and scleroproteins occurring in dystrophic hamsters is restored to normal following as little as 5 days of vitamin E therapy (Mauer and Mason, 1958) during this period necrosis associated with irreversible injury usually disappears.

These observations may bear some relationship to the findings of Aloisi *et al.* (1952) that in vitamin E deficient rabbits the myofibrils show either a loss of myosin or an alteration of the submicroscopic pattern in which myosin is organized prior to the loss of cross striations and other microscopic changes. There is also evidence (Corsi 1957) that in such muscles the myofibrils are reduced in number thinned and fragmented, and the extractible myosin significantly reduced in amount.

Attention has been given to other muscle proteins and to amino acids, especially in the dystrophic rabbit, in efforts to determine whether the loss of muscle mass in dystrophic muscle reflects impaired synthesis or accelerated breakdown of proteins, or an imbalance of both processes. An increased concentration of ribonucleic and deoxyribonucleic acids associated with increased urinary excretion of allantoin, in the monkey rabbit, and rat are interpreted as reflecting an increased rate of turnover of nucleic acids in dystrophic muscle (Young and Dinning 1951 Dinning and Day 1957) This phenomenon is demonstrated by following the incorporation of C formate into the nucleic acids of muscle in vitamin E deficient rats (Dinning, 1955) There is a decrease in glycine prior to the onset of gross dystrophy and, after dystrophy is well advanced, an increase in concentration of free

amino acids exclusive of the basic amino acids (Tallan, 1955 Smith and Nelson 1957) Of particular interest are the recent observations that cathepsin and dipeptidase activities are increased (Weinstock *et al.*, 1955 1956) These findings at least provide some important leads to the primary question which still remains unanswered.

A loss of muscle creatine and phosphocreatine, associated with increased output of creatine in the urine, is common to dystrophy of vitamin E deficiency and to many other conditions which result in general wasting or breakdown of muscle. There is evidence (Dinning and Fitch, 1958) that in vitamin E deficient rabbits there is increased synthesis and increased rate of turnover of creatine in the muscle as well as reduced ability of the muscle to retain creatine. In the rabbit, white muscle is said to show greater reduction in creatine, as well as more severe pathological lesions, than red muscle (Goettsch and Brown, 1932)

The degree of creatinuria, or the ratio of creatine to preformed creatinine excreted, provides a useful index of the severity of muscular dystrophy Since creatinine excretion usually remains relatively constant, the creatine/creatinine ratio will increase as dystrophy progresses. This ratio may also be increased somewhat through a diminution in creatinine excretion, especially when the dystrophic state is chronic and prolonged (Mackenzie and McCollum, 1941 Hove and Hardin, 1952) This latter phenomenon is thought to be related to the rather considerable loss of total mass of skeletal muscle tissue under such conditions. Increased creatine, and decreased creatinine, excretion are also characteristic features of E-deficiency dystrophy in the monkey (Dinning and Day 1957) and of choline-deficiency dystrophy in rabbits (Hove *et al.*, 1957)

Vitamin E therapy causes a prompt reduction in creatinuria with restoration of normal excretion pattern in 4 to 5 days, which is also the period during which irreversible injury usually completes its course. Too little attention has been given to the creatine status of the muscle itself following therapy In recent studies on deficient monkeys, Dinning and Day (1957) report that under conditions of therapy effective in bringing elevated concentrations of nucleic acids (RNA and DNA) to normal levels, muscle creatine is only partially restored to normal after several months of treatment.

It has been pointed out that dystrophic muscle is composed of muscle fibers showing varying degrees of sublethal injury nuclear dissolution,

necrosis and breakdown of cytoplasmic constituents, and also regenerative processes. It is therefore impossible to distinguish between those chemical abnormalities attributable to altered diffusion of electrolytes and other substances through the cell membrane, those due directly to intracellular disturbances of metabolism, and those attributable to actual disintegration of cellular constituents. The chemical changes observed are much like those of cortisone induced dystrophy (Milman and Milhorat, 1953) but differ in certain respects from those of simple atrophy or denervation atrophy of muscle (Hines 1952) They help in amplifying our picture of the dystrophic process even though they may, for the most part, represent secondary effects and provide no clue to the basic metabolic dysfunction in dystrophy (For a more complete discussion of this topic the reader is referred to the paper of Mackenzie, 1953.)

Briefly stated, chemical analysis of muscle after vitamin E deficiency reveals a variable decrease in potassium, magnesium, creatine and creatine phosphate, acid-soluble phosphorus, total nitrogen, myosin and actomyosin, glutamine, and glycogen there is, on the other hand an increase in sodium, chloride, ribo- and deoxyribonucleic acids collagen, cholesterol, and fat. There is considerable literature on altered levels of certain enzymes and enzymatic systems in both muscle and serum, and on possible functions of tocopherol in enzymatic processes (Nason *et al* 1957) Present information points toward a tendency for respiratory enzymes to increase and glycolytic enzymes to remain unaffected in dystrophic muscle, but offers little toward explaining the cause of dystrophy Studies on cathepsin and dipeptidase activities of muscle have been referred to earlier

2. *In Farm Animals*

a. Lambs An ailment of suckling lambs, most commonly referred to as "stiff lamb disease," has long been recognized as a disorder of considerable economic importance in the United States, Europe, and other countries. Affecting young lambs 1 to 5 weeks of age, it almost invariably appears in early spring just prior to the availability of green pasturage. Although Willman and associates reported in 1934 that the disorder could be produced experimentally by maintaining pregnant ewes on a diet composed mainly of cull beans and alfalfa hay and also recognized that in the skeletal muscles there were widespread lesions of a noninflammatory and nonneurogenic type, some years elapsed

before it was demonstrated (Willman *et al.*, 1945 Whiting *et al.*, 1949) that vitamin E had a prophylactic effect when fed to the ewes and a curative effect when fed to the affected lambs. Since then, essentially the same disorder has been produced with purified diets in liquid form (Culik *et al.*, 1951 Bacigalupo *et al.*, 1952) with and without the stress factor of tri-*o*-cresyl phosphate (Draper *et al.* 1952) The observations (Bacigalupo *et al* 1952) that, although the histological lesions in both disorders were identical, muscular weakness characterized the experimentally induced deficiency whereas muscular stiffness was the typical symptom of stiff lamb disease, suggest the possible influence of other factors in addition to lack of E in the naturally occurring disorder

There are interesting similarities between stiff lamb disease and late lactation paralysis in the rat. Symptoms appear during the suckling period, spontaneous recovery sometimes occurs, there is increasing difficulty in arising and walking the hind legs are more involved than the fore legs, there is creatinuria, and muscle lesions occur in the absence of symptoms Vitamin E therapy may abolish symptoms within a few days, but muscle lesions may persist for several weeks (Culik *et al.* 1951) Myocardial lesions are common. The symptomatology and histopathology of a myopathy of adult sheep commonly associated with a well recognized disorder known as "scrapie," have been described by Bosanquet *et al.* (1956), and reference is made to much of the recent literature on myopathies in this species. Their interpretations are that the myopathy in question is not related to lack of vitamin E, but is more of the nature of a myositis or a dermatomyositis.

b Calves A disorder known as "white muscle disease" or weisses Fleisch" in young calves has been recognized for many years in Europe, and more recently in this country It is an enzootic disease often causing serious losses in herds of beef cattle, but not affecting dairy herds. As in the case of "stiff-lamb" disease, the age of onset is early (3 to 10 weeks) the incidence is highest in early spring, the chief symptoms are those of muscular weakness, stiff gait, difficulty in standing suckling and swallowing, prostration, and rather sudden death usually attributed to cardiac failure Frequent association of the disorder with poor pasturage and improperly managed farms has indicated relationship to deficiency or excess of some dietary factor or factors. The first suggestion that a deficiency of vitamin E might be involved came from

Vawter and Records (1947) on the basis of a careful study of the symptoms and the gross and microscopic pathology observed in nursling calves showing spontaneous occurrence of the disease on cattle ranches in Nevada.

The first experimental production of the disorder on controlled diet, by Blaxter *et al.* (1952), was somewhat accidental, in connection with studies primarily concerned with nitrogen metabolism of young Ayrshire calves. Blaxter and his associates have since provided much valuable information concerning this disorder as it appears in the laboratory and in the field, and also a general review of the subject (Blaxter and Brown, 1952) Other experimental data have been recorded by Safford *et al.* (1954)

The lesions appear grossly as conspicuous, whitish areas of degeneration, are usually bilaterally symmetrical, involve particularly muscles of fixation, and are sometimes associated with edema and internal hemorrhage. Biochemically there is increased oxygen consumption (Blaxter *et al* 1952) and essentially the same alteration in the chemical components of muscle as observed in laboratory animals deprived of vitamin E namely an increase in ash, sodium calcium, fat, cholesterol, nucleic acid, stroma protein, collagen, and lipid phosphorus, and a decrease in dry matter potassium, total nitrogen, creatine, and iron, compared to normal muscle. The significance of these changes is discussed in some detail by Blaxter and Wood (1952) and the suggestion is made that the major biochemical defect in the muscle might be related to a decline in the synthesis of muscle globulins or an increase in the breakdown of these globulins.

As is true also of sheep, there are no features of the microscopic lesions of the skeletal muscles which would distinguish them from the lesions common to laboratory animals, except perhaps for a somewhat greater amount of calcification (MacDonald *et al* 1952) In both sheep and calves, the muscle lesions are markedly accentuated and often suddenly precipitated by oral ingestion of cod liver oil or other highly unsaturated fats, as is also characteristic of other herbivorous animals such as the rabbit, guinea-pig goat, and tree kangaroo In other varieties of kangaroo in captivity muscle lesions closely resembling those in laboratory mammals have been described and related to low serum tocopherol level (Schumacher and Schindler 1957)

c Avian Species In avian species there are striking species differences

in the manifestations of vitamin E deficiency during the early post hatching period. In chicks, the predominant symptoms are either exudative diathesis (accumulations of a serous-like fluid in subcutaneous tissues of the breast and elsewhere) or encephalomalacia (necrotic softening of the brain, secondary perhaps to alterations in related blood vessels) neither of which are observed in other avian species studied Skeletal muscle lesions, preventable by vitamin E and resembling but less extensive than those seen in ducklings, have been reported in chicks in association with encephalomalacia (Pappenheimer *et al.* 1939) and with exudative diathesis (Dam *et al* 1952) as well as in the absence of these symptoms (Machlin and Shalkop, 1956). There is marked variability in the incidence of lesions, which can often be recognized grossly by a whitish streaking of the muscle. Biochemical data are limited to observed increase in cholesterol but no significant decrease in creatine content of the muscle (Dam *et al.*, 1952) No very careful histological studies have been carried out, and no attention given to the response of established lesions to vitamin E therapy Cardiac and smooth muscle are unaffected.

Young ducklings reared for a few weeks on low-E diets exhibit a sudden onset of muscular weakness there is awkward gait with feet turned inward, difficulty in raising the head and in righting after being placed supine and eventual collapse (Pappenheimer and Goettsch, 1934 Pappenheimer *et al.* 1939) A similar disorder occurs under field conditions. At necropsy the skeletal muscles are pale, watery and translucent. Microscopically there is a widespread, acute type of necrosis with active regeneration, not unlike that described for late lactation paralysis in the rat. There is an increased water content and decreased creatine content of the dystrophic muscle. The failure of Victor (1934) to find an increase in oxygen consumption of the muscle, such as observed in dystrophic muscle of laboratory mammals and also of the chick, in which lesions are relatively mild, still lacks verification and explanation. As in the chick, cardiac and smooth muscle are unaffected, which is quite in contrast to the situation in turkey poults, where lesions are limited to a selective necrosis of the smooth muscle of the gizzard (Pappenheimer *et al.*, 1939)

B. Other Nutritional Deficiencies

1 *Vitamin C Deficiency*

Degeneration of skeletal muscle has long been recognized as a

feature of human scurvy though generally considered secondary to intramuscular hemorrhage, trauma, or cachetic atrophy However, about 30 years ago similar lesions were studied extensively in scorbutic guinea pigs and considered an intrinsic part of the disease (Höjer 1924 Meyer and McCormick, 1928, Dalldorf, 1929) Dalldorf calls attention to the high incidence of lesions in intercostal muscles (Fig 21) and states that placing scorbutic animals for an hour daily in a slowly rotating barrel, so that they are obliged to exert themselves to remain on their feet, produces florid lesions in muscles of the extremities. More recently, Murray and Kodicek (1949) and Boyle and Irving (1951) have given good descriptions of the lesions in guinea pigs The diets employed appear to have contained an adequacy of vitamin E.

The muscle lesions vary somewhat, depending upon the acuteness and chronicity of the scorbutic state, but have no features that clearly differentiate them from those of vitamin E deficiency in the guinea pig or rabbit. Some regeneration is usually evident, but the reparative response following vitamin C therapy has not been studied histologically Murray and Kodicek (1949) call particular attention to the associated edematous and hyperplastic state of the connective tissue of affected muscle and the impaired vascularization It is natural to wonder to what extent the lesions may be attributable to such changes in their supporting framework and to what degree they reflect loss of another important intracellular antioxidant which, because of its water-soluble properties, participates in metabolic functions of the muscle cell in a manner different from vitamin E.

2. *Other Vitamin Deficiencies*

Skeletal muscle is relatively immune to other vitamin deficiencies An extensive literature on the effects of specific vitamin deficiencies, other than those of vitamins E and C, in various animal species reveals no evidence of directly related lesions of skeletal muscle. This question has been put to a critical test in the rat by Mackenzie (1953) who employed a single basal diet with purified vitamin supplements adjusted such as to maintain a state of chronic deficiency of specific vitamins up to 16 to 20 weeks, followed by a more acute deficiency for the remainder of a 20 to 30 week period. Skeletal muscle was analyzed for creatine and chloride content and examined histologically Separate deficiency of thiamine riboflavin, pantothenic acid, pyridoxine, vitamin A, or protein had no effect on the muscles, except for an increased

chloride content after riboflavin deficiency On the other hand, when vitamin E was simultaneously excluded from the diets, muscles of rats deficient in pyridoxine, in vitamin A, or in protein showed more pronounced muscle lesions, a greater chloride content, and a lower creatine content than those of rats deficient only in vitamin E. Undernutrition was excluded as a complicating factor It has also been noted (Hove and Hardin 1952) that low protein accentuates creatinuria in rats on low E diets. The experiments referred to above also emphasize the importance in nutritional studies of assuring adequacy of all nutritional factors except the one under special study and the difficulty in properly evaluating and interpreting results obtained by different investigators employing diets of rather different composition for the production of specific deficiency states.

3 *Choline Deficiency*

Choline, a "lipotropic agent," has an important function in the phospholipid turnover in liver and kidney and in transport of fatty acids from the liver to the fat depots. It can furnish methyl groups for the synthesis of methionine, cystine, or creatine, and can serve as a precursor of acetylcholine. Deficiency of choline causes fatty livers and tubular necrosis of the kidney During the past 5 years, considerable attention has been given to lesions of the skeletal muscles and cardiovascular system which were apparently overlooked in earlier studies of the deficiency syndrome.

Hove and Copeland (1954) have called attention to the skeletal muscle lesions in choline-deficient rabbits. This was not an accidental observation but the result of experiments designed to test the hypothesis that deficiency of choline may interfere with acetylcholine synthesis which, in turn, may interfere with transmission of nerve impulses to the muscles or to their vascular supply The data obtained provided some support for this interesting hypothesis. After choline deficiency of 70 to 100 days, with more than an adequacy of vitamin E provided, rabbits show weakness, flaccidity and plasticity of the hind extremities such that when placed in unnatural positions they remain so for prolonged periods (Fig 22) The muscles are paler than normal histologically many fibers show swelling, contraction clotting loss of striations and necrosis, associated with some increase in connective tissue (Fig 23) The syndrome resembles that of chronic vitamin E deficiency in rabbits, yet high intake of vitamin E has neither a preventive nor cura

tive effect. On the other hand, choline therapy of a few days duration dispels all signs of creatinuria and muscle weakness as the authors state, "The legs could no longer be molded into bizarre positions, but instead had the feel of tightly coiled springs characteristic of normal rabbits." The observation that dietary choline lessened the vitamin E requirements of rabbits is also of interest.

Although Hove and Copeland (1954) indicate that muscular dystrophy of choline deficiency differs from that of vitamin E deficiency in that creatinuria appears more gradually and is associated with a decline in creatinine excretion, it may be noted that much the same picture has been observed in states of chronic vitamin E deficiency in rabbits (Mackenzie and MacCollum, 1941) and in rats (Hove and Hardin 1952) Mackenzie (1953) feels that the decline in creatinine excretion, which remains relatively constant in acute dystrophy merely reflects a more pronounced loss in the total mass of skeletal muscle in chronic dystrophy

In a subsequent report, Hove *et al* (1957) refer to damaged heart muscle and valves in their choline deficient rabbits, mentioning also that betaine hydrochloride at a 0.3% level in the diet prevented injury to skeletal muscle but not to cardiac muscle, whereas 0 12% choline chloride protected both. This may mean that skeletal muscle has a lower requirement for choline than cardiac muscle. It may be added that skeletal muscles of the betaine-supplemented rabbits were not entirely normal since they had a glossy translucent and greenish white appearance in the fresh state but were histologically devoid of lesions. It is also of interest that methionine deficiency also caused gross paralysis and extensive lesions in skeletal muscles, preventable by either methionine or homocystine. No reference is made to cardiac lesions in these animals.

In rats fed a low-choline, low protein diet for prolonged periods there have been observed extensive lesions of skeletal muscles which are said to differ morphologically from those characteristic of vitamin E deficiency (Aloisi and Bonetti, 1952) Other investigations of choline deficiency in the rat, and in the mouse also have made only casual reference to skeletal muscle injury but have reported rather extensive lesions of the cardiovascular system, as discussed later

4 *Anti-Stiffness Factor*

Guinea-pigs fed a milk diet supplemented with minerals and known

vitamins develop a peculiar wrist stiffness and skeletal muscle lesions of variable intensity and extent. There may also be considerable calcification, depending upon the inorganic constituents of the diet. Histologically the lesions (Fig. 24) somewhat resemble those of vitamin E deficiency (Harris and Wulzen, 1950; Mackenzie, 1953) but are not prevented by high vitamin E or accentuated by cod liver oil. Myocardial lesions also occur. Certain sterols such as ergostanyl acetate provide some protection. For further details, the reader is referred to the recent review by Krueger (1955). A similar calcinosis syndrome, with lesions of skeletal and cardiac muscle, occurs in cotton rats fed partially purified diets (Constant *et al.*, 1952).

5. *Potassium Deficiency*

Deficiency of potassium has a rather specific effect on cardiac muscle. However, lesions of skeletal muscles in the absence of myocardial injury are reported in dogs fed a potassium deficient diet which, when fed to rats, produces only myocardial lesions (Smith *et al.*, 1950), again illustrating species differences in response to the same inadequacy of diet. The syndrome resembles familial periodic paralysis of man. A series of mild attacks are usually followed by a progressive paralysis and death due to respiratory failure. No lesions are found in the central nervous system. The disorder can be reversed by potassium therapy. The muscle lesions are widely scattered and characterized chiefly by contraction-clot necrosis with regenerative reactions; their somewhat minor character in proportion to the striking muscular weakness and disability of the animal suggests widespread functional impairment of muscle fibers in the absence of much structural change.

III. CARDIAC MUSCLE

Cardiac muscle differs from skeletal muscle in certain structural, biochemical and physiological features. Hence it is of interest that it reacts to vitamin E deficiency in much the same manner as does skeletal muscle, yet responds quite differently from skeletal muscle to deficiencies of choline and potassium.

A. Vitamin E Deficiency

Myocardial lesions and electrocardiographic abnormalities have been observed in many animal species subjected to vitamin E deficiency. Usually they occur in association with the skeletal muscle lesions and

are accelerated or accentuated in much the same manner by dietary unsaturated fats. The range of response of cardiac muscle is even more limited than that of skeletal muscle, that is, the pattern of cellular response is qualitatively the same and lesions differ chiefly in the extent of myocardial involvement. The lesions occur as a myocardial necrosis (Fig 25) in which there is observed a progressive vacuolation, loss of myofibrillar structure, nuclear pyknosis, and dissolution and gradual disintegration of cardiac muscle cells in isolated or confluent zones of the myocardium (chiefly ventricular and interventricular, but often auricular as well) associated with the presence of a few macrophages which do not actively invade the injured cells but assist in removal of the breakdown products. Edema and small hemorrhages are sometimes seen. Purkinje fibers are usually not involved. There is connective tissue replacement of the damaged muscle cells, for regenerative reactions are not observed in cardiac muscle.

In the rabbit, the laboratory animal most extensively studied, there is increased oxygen consumption of the cardiac muscle (Gatz and Houchin, 1951) normal glycogen concentration, but marked reduction in creatine phosphate (Mulder *et al.*, 1954) and electrocardiographic abnormalities consisting of right axis deviation, notching of T and T_1 waves and inversion of T_1 (Gatz and Houchin, 1951) The latter investigators, and also Bragdon and Levine (1949) give detailed descriptions of the myocardial lesions. Similar lesions observed in the rat, differing chiefly in the presence of considerable acid-fast pigment in muscle cells and macrophages, are not significantly modified by many months of vitamin E therapy (Mason and Emmel, 1945)

Cardiac lesions and electrocardiographic abnormalities are particularly pronounced in herbivorous farm animals suffering from inadvertent or intentional depletion of vitamin E. They are considered responsible for sudden death in cattle on low E diets (Gullickson and Calverley 1946) in calves manifesting "white muscle" disease (Vawter and Records, 1947, Blaxter *et al.*, 1952) and in "stiff lamb" disease (Willman *et al.*, 1934 Culik *et al* 1951 Draper *et al.*, 1952) Correlations between lesions and creatine content are not as impressive as for skeletal muscle (Blaxter *et al.*, 1952)

B. Vitamin B Deficiency

It is well recognized that thiamine deficiency alters the function of cardiac muscle and also produced typical myocardial lesions in a

variety of animal species (swine, rats, dogs, foxes) The lesions in swine, which are quite extensive, have been described in detail by Follis *et al* (1943) They are in no way unique, except perhaps for more involvement of the auricular muscle than observed in most nutritionally induced injuries of the myocardium, which may bear some relationship to a significantly reduced oxygen consumption observed in the auricular but not in the ventricular muscle in thiamine deficiency The reader is referred to the monograph of Follis (1948) for a more complete discussion of this subject.

C. Choline Deficiency

The cardiac musculature of the rat is peculiarly susceptible to choline deficiency especially when dietary fats are high. Kesten *et al* (1945) observed sudden death from heart failure during the first week of feeding choline deficient diets high in ethyl laurate, and describe a widespread interstitial myocarditis most marked in the subendocardial regions but usually not involving the conduction system. There was mild edema, considerable cellular infiltration and variable amount of necrosis of heart muscle cells. The possibility of potassium deficiency which results in a similar type of lesion, was excluded.

According to Wilgram *et al* (1954) and Wilgram and Hartroft (1955) the myocardial lesions are accentuated or intensified by many other types of dietary fats, are initiated by the appearance of considerable lipid in the cardiac muscle cells before swelling and necrosis occur are always associated with a fatty liver are not seen in the absence of a fatty liver and are usually associated with lesions in the larger blood vessels. The latter begin with lipid accumulations in the endothelium and intima followed by medial necrosis and variable degrees of calcification. Fat released following lysis of cardiac muscle cells is taken up by macrophages which disappear subsequent to healing and replacement fibrosis. It is also of interest that male rats are much more susceptible than females, and that the natural resistance of the female rat is greatly reduced by treatment with androgens and growth hormone.

In mice myocardial lesions are less readily produced by choline deficiency than in rats, are accentuated by marginal intake of proteins providing sulfur-containing amino acids, and apparently do not show the sex differences observed in rats (Williams and Aronsohn, 1956 Meader and Williams, 1957)

D Potassium Deficiency

Of the various inorganic constituents of the diet, only potassium seems to be indispensable for maintaining the integrity of muscle this relationship pertains almost exclusively to cardiac muscle and appears to be linked in some manner with certain of the B vitamins. Attention has been called to effects of thiamine deficiency on the myocardium. Thomas *et al* (1940) present evidence that cardiac lesions occur in the rat only when there is a simultaneous deficiency of potassium and an inadequacy of B vitamins, particularly B_1 Of particular interest are the observations of Follis (1942) that in the rat a combined deficiency of potassium and thiamine, each of which results in cardiac lesions if absent from the diet, produces no cardiac lesions but results in lesions of skeletal muscle which are not produced by single deficiency of these factors. It has also been reported (Smith *et al.*, 1950) that a potassium deficient diet producing rapid myocardial necrosis in the rat causes only lesions of skeletal muscles in the dog Follis (1948) to whom the reader is referred for further details considers that the myocardial lesions of potassium and of thiamine deficiency are histologically rather different from those of vitamin E deficiency in the rat.

E. Tryptophan Deficiency

Among the specific amino acid deficiencies studied, muscle lesions have been noted only after tryptophan deficiency in rats. These involve only the cardiac musculature according to Scott (1955) although a separation and shredding of fibers in both cardiac and smooth muscle has been reported by others (Adamstone and Spector 1950) Scott describes cytoplasmic vacuolation and karyolysis followed by cloudy swelling and hyalinization of cardiac muscle fibers, with the usual connective tissue replacement and formation of myocardial scars.

IV SMOOTH MUSCLE

A. Vitamin E Deficiency

Smooth muscle is highly resistant to alteration by nutritional deficiencies, with the single exception of vitamin E deficiency After several months of vitamin E deficiency rats exhibit a yellowish discoloration of the uteri which gradually increases to a brownish color due to the accumulation of brownish fluorescent, acid-fast, insoluble

inert pigment granules in smooth muscle cells (and in connective tissue macrophages) of the myometrium (Fig 26) a similar but somewhat less pronounced change is seen in the smooth muscle of the fallopian tube, seminal vesicle, prostate, vas deferens, ureter splenic capsule and trabeculae, bronchi, and small intestine, and occasionally in larger blood vessels (Martin and Moore, 1939 Mason and Emmel, 1945) The pigment, sometimes referred to as "ceroid," is considered to be either a lipoprotein or a polymerization product of fatty acids possessing at least eighteen carbon atoms and two or more unsaturated bonds (Filer *et al.*, 1946)

Pigment granules, appearing first at each pole of the nucleus of smooth muscle cells, gradually accumulate and push the myofibrils peripherally Lindner (1957) on the basis of electronmicroscopic observations, thinks that there is a reduction in the number of myofibrils and that the membrane system of mitochondria possibly participates in the formation of pigment granules. Eventually there is distension and even distortion of the muscle cells, but it has not been proven that actual necrosis of the cells occurs nor is it clear whether the pigment present in macrophages in close proximity to the muscle cells is derived from them, with or without necrosis of the cell. There is an obvious relationship between the metabolic activity of smooth muscle cells and the accumulation of pigment, for it does not appear if deficient rats are ovariectomized before puberty but does so if such rats are given estrogen treatment (Atkinson *et al.* 1949) Intracellular accumulation of the pigment appears not to significantly impair physiological activity of the muscle It is somewhat reduced but not eliminated following prolonged vitamin E therapy pregnancy castration, or hormone treatment.

There are interesting species and organ differences with respect to the site of accumulation of pigment, which must reflect rather marked differences in metabolism of smooth muscle, especially in content and utilization of lipids. In the monkey pigment is especially marked in the smooth muscle of blood vessels (Fig 27) and occurs also in that of the *small intestine, gall bladder urinary bladder* and bronchi (*Mason and* Telford, 1947) In the Syrian hamster pigment is quite abundant in blood vessels, urinary bladder and intestine but absent from the uterus. In the mouse and dog it is limited largely to the uterus and the intestinal muscular respectively Pigment cannot be demonstrated in smooth muscle of the vitamin E deficient rabbit, guinea-pig, or Florida

cotton rat (Mason, unpublished studies) and has not been mentioned in reference to deficiency states in herbivorous farm animals or avian species.

On the other hand, a different type of effect of vitamin E deficiency on smooth muscle has been noted in avian species. In turkey poults, and to a lesser degree in chicks (but not in ducks, which show only skeletal muscle lesions) there is described a patchy hyaline necrosis restricted to the smooth musculature of the gizzard, associated with slight inflammatory reactions and replacement fibrosis (Jungherr and Pappenheimer, 1937 Jungherr, 1949)

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CHAPTER VII

Aging Changes in Muscle

L. J. Rubinstein

I. INTRODUCTION

A review of histological changes due to aging must take into consideration the general distinction between senescence as a property of the living cell and senility as manifested by the alterations to which a multicellular organism is subject in advanced life. In respect of the latter it is widely held that the aging process cannot easily be divorced from those functional and pathological changes, affecting tissues and organs, with which old age is commonly associated. On the other hand the notion of senescence postulates, when applied to the cell, a particular process in which intracellular enzyme activity, metabolic exchanges, and modifications in the colloidal nature of the protoplasm are in all probability implicated.

According to Cowdry (1952) the more general aging changes in mammalian cells consist in alterations in the nucleocytoplasmic ratio, a decrease in active cytoplasm, and modifications in the water content. It is evident that these changes must be difficult to estimate in those cells which, as in the basal layer of the epidermis, for example, have a short individual life and the property of continuous reproduction: they would, on the contrary, be expected to develop in those which lack this attribute (Cowdry's "fixed post-mitotic cells"). These include neurons and, perhaps less absolutely, skeletal and cardiac muscle. A considerable literature has accordingly been devoted to senile changes

in the neurons. The relative paucity of similar studies on skeletal muscle cells is perhaps partly due to the great difficulty of distinguishing between alterations due to aging and degenerative lesions secondary to diseases of the cardiovascular nervous, or locomotor system, or to disuse The difficulty is enhanced by individual differences in body build and nutritional state, and by the divergences which may be expected to exist between muscles in various parts of the body both in regard to their histological structure and metabolism, and to the amount of work they have been called to perform during life Consequently the more significant and interesting studies have been devoted to changes in the external ocular muscles, in which secondary general pathological changes and individual variations can, it is assumed be reduced to a minimum, and which are subject throughout life to a constant degree of activity Furthermore, they like a few others in the body undergo a series of pronounced morphological alterations which are not demonstrated in the larger limb and trunk muscles Generally speaking the cells in the latter as well as in cardiac muscle preserve in the absence of disease a remarkable degree of structural integrity throughout adult life

II. MORPHOLOGICAL CHANGES IN AGING MUSCLE CELLS

A Changes in Size of Muscle Fibers and Their Nuclei

In both skeletal and cardiac muscle there is a progressive decrease in the nucleocytoplasmic ratio during growth, but this apparently simple relationship becomes more complex when the number of nuclei per muscle fiber as well as their respective sizes, are taken into account. In an analysis of these relationships on material from 9 human subjects, Schiefferdecker (1911) found that in the diaphragm the proportion of *total* nuclear to cytoplasmic mass is highest in the 5-month embryo and lowest in the newborn it again increases slightly in the adult. On the other hand, the simple ratio between cytoplasmic and nuclear size increases throughout life The individual nuclei were also appreciably larger in the embryonic muscle than in the newborn and in five of the six adult subjects he examined. The interest of these observations is somewhat limited by the restricted material, the oldest subject being 64 years of age In a parallel study of 18 hearts from patients ranging from 1 to 77 years Schiefferdecker (1916) found that the number of nuclei relative to the fibers is highest in children and gradually de-

creases with advancing age. There is a gradual increase in the absolute size of the muscle fibers as measured by their diameters and, in contra distinction to subsequent observers, Schiefferdecker found the largest fibers in his oldest subject. As a corollary there is an increase in the ratio between cytoplasmic and nuclear volume, although, as Schieffer decker's data indicate, the absolute nuclear mass remains the same or may even increase. An increased nuclear volume associated with hyper chromatism has also been noted in cardiac muscle by Lansing (1952)

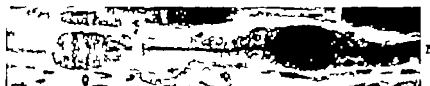
More information on changes in the caliber of cardiac muscle fibers was obtained by Dogliotti (1931) in a histological study of 200 hearts from patients up to the age of 93 He reported a gradual increase in the diameter of the muscle fibers with advancing age, starting from 7 to 8μ in the newborn up to 20 to 25μ in the third decade. No further general increase, apart from individual variations, was recorded above the age of 30 but after the seventh decade there was a slight tendency for the caliber of the muscle fibers to decrease. These observations were broadly confirmed by Tormene (1953) who however, found that the muscle fibers increased in size until the age of 50 There are also many individual variations, unrelated to the weight of the heart, or to sex.

In extreme old age, this decrease in fiber size may lead to a simple senile ("brown") atrophy a condition relatively seldom observed by the pathologist in its pure state, i e. in the absence of wasting disease Thus Warthin (quoted by Korenchevsky *et al.*, 1950) noted that in 38 years experience he could only select out of a great number of autopsies of old subjects 25 cases who died of senility alone, death being apparently caused by simple myocardial atrophy and inadequacy with little or no coronary sclerosis.

In senescent female rats, Korenchevsky *et al* (1950) found on com parison with younger controls a definite atrophy of the cardiac muscle but a subsequent investigation (Korenchevsky *et al.*, 1953) in male senescent rats revealed in addition to the atrophy an increased number of large muscle fibers, which these authors regard as a focal hyper trophy compensating for the degeneration and consequent loss of the atrophied elements.

Aging changes reflected in the caliber of fibers in the larger trunk and limb muscles have not attracted much detailed attention. The so-called B fibers, which Wohlfart (1937) designates certain isolated larger fibers in the primary bundles of many voluntary muscles of the embryo and the infant have seldom been distinguished by him above


A


B


C


D


E

the age of 12 At the other extreme, it is generally accepted (Adams *et al* 1953) that a muscle atrophy takes place in old age but that it is highly variable both in individual cases and in respect of different muscle fibers within a single histological sample. Sarcolemmal nuclei are sometimes increased in number but increases of intracellular pigmentation and of interstitial connective tissue are usually considered to be more characteristic. Quantitative data on the size of the large skeletal muscle fibers in so-called "senile atrophy" are, however lacking An atrophy of the skeletal musculature has also been postulated in the senile rat (Lowry *et al.*, 1942) but this inference is based less on morphological than on chemical data. These will be touched on in a subsequent section of this chapter

In the external ocular muscles, Bucciante and Luria (1934) measured the maximal and minimal diameters of 500 fibers from the superior rectus in 25 cases ranging from 14 to 92 years. They recorded a definite increase in the average diameters from about 16μ from the ages of 14 to 40 to about 23μ at 80 or over but these differences only became marked above the age of 80 They were matched by an increase in the incidence of the largest diameters. Their measurements were, however, subject to a great deal of individual variation and it is clear from their tables that there is a notable increase in inequality of fiber size in this muscle above the age of 60 This is illustrated in Fig 2, from the lateral rectus muscle of a man of 83 It suggests that some of the larger fibers may represent a compensatory hypertrophy secondary to the degenerative changes in the remainder

B. Morphological Changes in External Ocular Muscles

For reasons already stated, these changes have attracted particular

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Fig. 1 Aging changes in external ocular muscles (longitudinal sections)

A. Pla like arrangement of myofibrils. Lateral rectus, male of age 41 Magnification × 550. Phosphotungstic acid hematoxylin (P T.A.H)

B. Tangled myofibrillary balls. Preservation of cross-striations in residual central myofibril (Superior rectus, male of age 79 Magnification × 350. P T.A.H.)

C. "Ringbinde " Condensation of myofibrils with bottle-brush appearance on the left (Superior rectus, male of age 79 Magnification × 480. P T.A.H.)

D Central condensation of myofibrils (top fiber) with increased peripheral sarcoplasm the myofibrils are breaking up on the right. Myofibrillary ball (out of focus in lower fiber (Lateral rectus, male of age 41 Magnification × 450. P T A.H.)

E. Disappearance of myofibrils and granular degeneration of swollen sarcoplasm. Lateral rectus, male of age 83 Magnification × 360 P T.A.H)

attention They were first described in detail by Buccianite and Luna (1934) and subsequently confirmed by Wohlfart (1938) They consist above all in alterations in the disposition of the myofibrils culminating in their disintegration, and in an apparent increase of sarcoplasm. Various aspects of these modifications are illustrated in Figs. 1 and 2. It must be emphasized that the changes about to be described are not restricted to the elderly but that they are also found, though in their incipient stages mainly and to a considerably lesser extent altogether in the eye muscles of young subjects. Their earliest manifestation lies in a close intertwining of obliquely running myofibrils, thus giving to

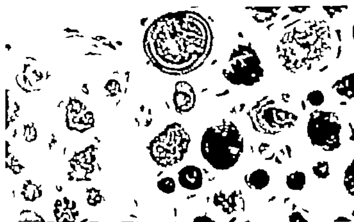


Fig 2 Aging changes in external ocular muscles (transverse section) "Ringbinden periphal sarcoplasmic masses and central condensation of myofibrils loss of myofibrils and granular degeneration of sarcoplasm in one fiber (top right corner of figure) great inequality in size of muscle fibers. (Lateral rectus, male of age 83. Magnification 490 slightly reduced. P T.A.H.)

the fiber a plait like appearance (Fig 1A) Another early change is the appearance at the periphery of the fiber of irregular fringes of faintly granular fibril free sarcoplasm. In a more advanced stage, these increase in thickness and uniformity they often contain an enlarged subsarcolemmal nucleus with a conspicuous central nucleolus. Later still thick homogeneous masses of peripheral sarcoplasm are seen both in transverse and longitudinal sections surrounding a central core of condensed residual myofibrils which are frequently coarsened (Fig 2) Simultaneously with this apparent central retraction, the myofibrils undergo the most diverse changes in their alignment they

may form irregular plexuses, which break up in various directions within the sarcolemmal sheath (Fig 1D) they may be condensed in skeins or tangled fibrillary balls sometimes scattered at regular intervals along the course of a fiber but retaining a single centrally located myofibril (Fig 1B) or in places, the thickened residual central fibrils adopt a cork-screw arrangement or exhibit closely set barbs, giving them on longitudinal section a bottle brush appearance (Fig 1C) All transitional forms between these various aspects may be met.

One of the most characteristic forms of myofibrillary derangement is the so-called "Ringbinde," or striated annulet, first described by Heidenhain in 1918 and originally regarded as pathognomonic for dystrophia myotonica. They have been frequently described since, particularly by Wohlfart (1938) and Bergstrand (1938) Thus the myofibrils adopt within the muscle fiber a spiral course which is perceived in longitudinal sections as a concertina like deployment of a single myofibril (Fig 1C) but is best revealed in transverse sections as a circular or concentric striated coil (Fig 2) Figures of eight may also be formed and transitional stages between plaitlike formations and fully developed "Ringbinden" may be identified. A noteworthy feature of these diverse alterations is the fact, illustrated in Fig 1B and 2 that striations remain preserved in the individual fibril to a remarkable degree This integrity is found not only in the "Ringbinden" but also in the most intricately entangled myofibrillary balls.

Lastly evidence of a progressive structural degeneration of myofibrils—loss of definition and of cross-striation—culminating in their disintegration and complete absence, is found to a varying extent. This degeneration starts usually at the periphery of the fiber and involves the centrally situated myofibrils last. This results in large isolated masses of granular or floccular sarcoplasm (Figs. 1E and 2) frequently containing lipofuscin pigment. The deposition of this pigment takes place in the external ocular muscles *pari passu* with these structural alterations and is often found in the early stages in the peripheral sarcoplasmic seams already mentioned. There is also a progressive increase of elastic connective tissue.

The changes which have been described are observed with particular frequency in the superior and lateral recti, and in the superior oblique muscle. They are usually present to a much lesser degree in the levator palpebrae superioris. Their incidence varies from case to case. Isolated instances may as already noted, be seen in young people, and

"Ringbinden" may appear in the third decade. Wohlfart (1938) states that he has seen an example of the latter in the superior rectus of a child of 8. In general, however, these changes are lacking in many specimens from young subjects of 10 to 22 years (Bucciante and Luria, 1934) and show a definite increase in frequency and complexity with advancing age. They are often well developed in the fifth decade and highly abundant in the eighth and ninth. According to Wohlfart (1938) the B fibers, which are said to remain discernible in the external ocular muscles throughout life, tend not to share them.

Similar alterations have also been found in a few other selected muscles in the body in the absence of disease: thus Malan (1934) has recorded myofibrillary changes and "Ringbinden" in the tensor tympani of the aging dog and cat, restricted, however, to the "large fibers," which account for 70 to 80% of the fibers in this muscle. Bucciante and Luria (1934) have observed "Ringbinden" in the internal laryngeal muscles of a man aged 79, and Bach *et al.* (1941) have described and illustrated changes in the abductor muscles of the larynx very suggestive of myofibrillary tangles: it has been submitted that the changes in these muscles may be related to the altered character of the senile voice.

Of special interest is the incidence of similar modifications in myotonia dystrophica, namely the "Ringbinden" and the peripheral sarcoplasmic masses (Wohlfart, 1951; Greenfield *et al.*, 1957). As already noted, the "Ringbinden" were originally believed to be pathognomonic for this condition. They are, however, also encountered in other forms of muscular dystrophy. The occurrence of these changes as part of the normal physiological process of "aging" therefore calls for caution in the assessment of their pathological significance when external ocular muscles are examined. They are, however, only seldom found in the normal aging skeletal musculature apart from the muscles already specified. Greenfield *et al.* (1957) have noted "Ringbinden" in a biopsy from normal temporalis muscle and Bergstrand (1938) has recorded them in the diaphragm of a healthy man of 53.

It has sometimes been stated that these appearances, in particular the "Ringbinden," are the result of artifacts due either to post mortem contraction of the myofibrils or to fixation (Adams *et al.*, 1953). Such a view appears disproved by (1) their curiously selective though fairly constant distribution, (2) their quantitative increase in aging, (3) their presence in pathologically affected muscles, (4) the presence of

lipochrome pigmentation in the degenerate areas (5) their demonstration in specimens where intra-orbital irrigation with formalin has been carried out immediately after death (6) finally their presence, in the fresh unfixed state in teased preparations (Bucciante and Luria, 1934)

The significance of these morphological alterations, apart from their apparent though unexplained association with "aging" their mechanism, and the reason for their restricted topographical incidence are obscure. The presence of similar changes in myotonia dystrophica where an excessive contractility of the myofibrils is associated with an excessive tonus of the musculature and atrophy is also imperfectly understood Wohlfart (1951) has suggested that the "Ringbinden" might be ascribed to decreased elasticity and partial elongation of the myofibrils, whereby they gradually arrange themselves in annular or spiral coils, and Greenfield *et al* (1957) have recalled that excessive sarcoplasm may be related to the rate of contraction of the muscle fibers It has been stated (Schiefferdecker 1911) that the external ocular muscles, as well as the laryngeal muscles and the diaphragm have a higher oxygen content than any other skeletal muscle. These changes may therefore perhaps be linked with the increased performance, the rapid contraction rate and the higher metabolic activity of these muscles, though this is entirely speculative. Furthermore, the remarkable paucity of physiological ill-effects, as manifested in elderly individuals, of such extensive morphological alterations remains unexplained.

C. "Wear and Tear" ("Aging") Pigment

Reference has already been made to the increased deposition of "wear and tear" pigment as an aging process. This pigment, one of the "lipofuscin" group, ranges in cardiac and skeletal muscle from a golden yellow to brown in microscopic preparations. It is weakly to moderately basophilic, has a variable affinity for Sudan stains in paraffin sections, but is insoluble in lipid solvents. It is usually acid fast, and often exhibits feeble reducing properties. As an aging phenomenon it has been studied most extensively in ganglion cells (for references see Sulkin 1953) but is also found in other sites, particularly the liver adrenals, testis, and epididymis. There are however several histochemical differences between the lipofuscins in these various organs.

In muscular tissue it is best observed in the heart, where it has been studied particularly by Dogliotti (1931) and by Jayne (1950) Here it

presents in the second decade as fine single granules or small clusters at either or both poles of the nuclei later these granules coarsen and extend laterally into the cytoplasm They also appear separately in other parts of the muscle fibers in older subjects The amount, size and distribution vary both in the muscle cells and in individuals of the same age and sex, but there is on the whole a progressive increase with age both in the number of cells affected and the amount of pigment in each. It is nearly always abundant above the age of 70 (Fig 3)



Fig 3 Lipofuscin pigment in myocardium. (Male of age 79 Magnification 330 slightly reduced. Long Ziehl-Neelsen)

A parallel increase of pigmentation is seen in the external ocular muscles here intracellular pigment may already be demonstrated in healthy subjects in the third decade and is usually fairly pronounced in the fifth Bucciante and Luria (1934) have noted its presence at the early age of 13 Such a high degree of pigment deposition is not encountered in the larger limb muscles, where granules only begin to appear at either nuclear pole over the age of 60 and are obvious only in extreme old age

The chemical nature of the pigment in muscle has long been disputed Because some of it was said to give a positive iron reaction it was regarded (Conner 1928) as partly mixed with hemosiderin and assumed to be a product of metabolism from hemoglobin or in the case of muscle from myoglobin It has also at times been equated by older authors with the iron-free pigment "hemofuscin" found in hemochromatosis in association with hemosiderin. Most of it, by com

mon consent, contains no iron, and it is now widely accepted as having a chemical relationship to the "ceroid" found in the liver and the "lipochrome" pigment of the nervous system Pearse (1953) considers that the "lipofuscins" include in a wide spectrum a group of histochemically reacting substances which range according to the stage of maturity of the pigment from a sudanophilic precursor at one end to a fully oxidized product with a negative affinity for fat stains at the other Jayne (1950) found that the cardiac pigment gave a positive Millon test and regards it as an unsaturated lipid bound to a protein of high molecular weight. Like lipofuscins elsewhere, it gives a negative reaction for tryptophane (C.W M Adams, 1957 personal communication) Further details on the histochemical reactions of the cardiac muscle pigment and its divergences from related pigments elsewhere will be found in Pearse (1953) and Lillie (1954)

The significance of this deposition is still uncertain and the designation of "wear and tear pigment," though useful is uninformative. It is generally considered to be related directly to the cellular aging process (Lansing 1952) and probably constitutes a by product of cell metabolism Its identity with the pigment which appears in vitamin E-deficient animals has been canvassed by several authors (see Adams *et al.*, 1953) Though commonly linked with "atrophy " it is, perhaps significantly found normally in those muscles (cardiac, ocular) which have been the most constantly active throughout life. It is also of some interest that Kny (1937) in his study of several limb muscles (sternomastoid, subscapularis, sartorius and gracilis) observed a greater amount of pigment in strong and active muscles than in those which were paralyzed and atrophied and likewise, more of it was noted in muscles of movement than in those which maintain posture.

The rare incidence of excessive pigmentation in the myocardium of younger subjects and its occasional paucity in the elderly is unexplained. Watjen (1940) has reported the curious case of a 14-month-old infant with multifocal chronic sepsis, who showed a considerable deposition of lipofuscin in the muscles of the trunk, limbs, larynx, and tongue, and several examples are now on record in which an excess of similar pigment was found in the gastrointestinal musculature of patients with a disturbance of intestinal absorption such as spruelike syndromes and fibrocystic disease of the pancreas (Ansanelli and Lane, 1957) It seems therefore that the relation of this pigment to the aging process needs further clarification

III. CHANGES IN WATER AND ELECTROLYTE CONTENT

A number of quantitative alterations in the water and electrolyte content have been recorded in aging skeletal and cardiac muscle. Contrary to the usual belief which associates senescence with a progressive dehydration, evidence has accumulated indicating an increased water content in the skeletal musculature, in particular in the extracellular spaces. It may be useful briefly to compare these changes with those taking place during growth.

During the period of growth, the total concentration of water and of sodium and chloride electrolytes in muscle tissue diminishes, whereas there is a rise in the concentration of intracellular ions roughly commensurate with the relative increase in the number of cells. These alterations have been recorded in the skeletal muscle of the rat, the cat, and the chicken (for references see Lowry and Hastings, 1952) and have been interpreted as due to a loss of extracellular fluid accompanied by only minor variations in the ionic constitution of the intra and extracellular compartments the loss of extracellular fluid is attributed to the reduction in interstitial space consequent on the increase in size and number of the individual muscle fibers. Similar changes associated with growth have been recorded by Hastings *et al* (1939) in the myocardium of dogs.

The available data suggest that a reversal of this process takes place in aging Simms and Stolman (1937) in a chemical analysis of the psoas muscle in a limited material (17 subjects) of homicidal and accidental deaths, found in the aged an increase of total sodium, chloride, and calcium, a slight increase in water and a decrease in potassium, magnesium, phosphorus, nitrogen and ash. Similar changes were observed in the kidney liver and spleen. These results were interpreted by Lowry and Hastings as due to an increase in the extracellular fluid compartment. This view gained support when the actual figures were compared with the expected water and electrolyte increases obtained on the basis of changes in the sodium levels only These findings have been confirmed in the skeletal and cardiac muscles of the senile rat (Lowry *et al* 1942) on the other hand, the intracellular concentrations of water phosphorus, and potassium underwent no significant modification. The changes appeared closely comparable to those obtained by Hines and Knowlton (1937) in the gastrocnemius of the rat following denervation atrophy here the increases of chloride

and calcium concentrations were regarded as matching the extent of the atrophy and the relative increase in connective tissue. Lowry and Hastings (1952) summarize these findings by advancing the thesis of an extracellular edema occurring in old age, which could be consequent to atrophy loss of tissue cells, or even to cardiac or renal hypofunction, but they cautiously add that similar changes are also met in muscular atrophy due to other causes.

Histological examination of large skeletal limb muscles in aging individuals often reveals a varying increase in extracellular space, especially between the primary muscle bundles, which contrasts with the compact arrangement of the muscle bundles in young healthy subjects. That such appearances are not solely due to an artifactual retraction, after fixation, of the sarcoplasm from the endomysium can be established by staining the latter for reticulin. But a conspicuous degree of individual muscle fiber atrophy is usually not evident microscopically in normal subjects, even in extreme old age, if care is taken to exclude samples from lower limb muscles of bedridden patients. On the other hand possible changes in the extracellular compartment may well have to be related to other factors, such as local tissue edema, congestive heart failure, renal impairment, prolonged recumbency nutritional or disuse atrophy

Some attention has also been paid to changes in the calcium content. It is known that calcium tends to increase with age in various organs (Lansing 1952) in particular in arteries, in elastic tissue, and in the heart (Barnes, 1942) and it has been suggested that this rise is part of a general trend for calcium to shift from the bones into the soft tissues. Lansing (1942) found an increase of calcium in the gastrocnemius of the aging toad concentrated along the surface of the cell membranes a finding which he associates with a lower permeability of the latter

IV EFFECTS OF AGING ON INTERSTITIAL TISSUE

A. Elastic and Collagen

In human cardiac muscle, Miller and Perkins (1927) recorded a progressive increase with age in the number of elastic fibers, a finding subsequently confirmed by Dogliotti (1931) who noted however a great deal of variation from case to case. But he found no increase of collagen. There is therefore no evidence to support a so-called "fibrosis of old age" as distinct from the focal fibrosis so often met as the result

of small old infarcts. Bacon (1948) who studied the reticulin fibers of the myocardium of the mouse, has likewise found no increase in aging

In voluntary muscles, the observations of Bucciante and Luria (1934) indicate a fairly constant increase in number and thickness of the elastic fibers in the external ocular muscles, where, as already noted by Schiefferdecker (1911) they are considerably more abundant than in skeletal muscles elsewhere. There is also a progressive increase in interstitial collagen in the superior rectus apparently unrelated to any change in the size of the muscle fibers. In the sternomastoid any increase in elastic fibers is according to Bucciante and Luria, so feeble as to be of no importance, but there is a slight increase of collagen, rather more apparent above the age of 60 when it may sometimes be secondary to a focal atrophy of the muscle fibers The perimysium is also more abundant An increase of collagen was also found in the musculature of the tongue

B Adipose Tissue

Frantzell and Ingelmark (1951) have investigated in some detail the incidence and distribution of fat in human muscles at various age levels by correlating morphological with radiological and chemical findings. They examined microscopically the gastrocnemii in 175 necropsies ranging in age from birth to the age of 80 and also the biceps brachii in a third of these cases. They relied on thick frozen preparations (30 to 50μ) of longitudinally and transversely sectioned muscle stained with Sudan III This technique, while revealing very little intramuscular fat in the newborn demonstrated a progressive increase with age of perivascular and interstitial adipose tissue, which was matched by a parallel increase in amount and frequency of fat immediately under the epimysium ("subfascial fat") By the fourth decade, perimysial fat was present in sufficient amounts to separate the primary muscle bundles and produce a honeycomb pattern in microscopic preparations Central areas of degeneration" were often observed in subjects over 50 when fatty replacement of muscular tissue was extensive Where comparison was possible, the gastrocnemius always showed larger amounts of fat than the biceps brachii, the differences being particularly marked above the age of 30 These changes were partly confirmed by chemical estimation of the fat content of muscles in the gastrocnemius there was a gradual increase from 6 to 8% of the dry weight at birth to about 33% by the eighth decade, but the biceps

brachii showed no corresponding increase. It is of interest that fat could only be demonstrated histologically when it exceeded 7% of the dry weight of muscle tissue hence it was estimated that this figure represents the proportion of intracellular fat. Unlike subcutaneous fat intramuscular fat did not display a definite sex preference, although the general impression was gained that it was slightly higher in females. Radiological investigation by a special technique of 222 healthy subjects confirmed the increased incidence of interstitial fat with age, greater amounts being observed in females than in males, and in the leg than in the thigh muscles. Subfascial fat, which increases with age at first, seemed to remain fairly constant above the age of 55 and no definite correlation between the amounts of interstitial and subcutaneous adipose tissue was found nor was the latter associated with aging The authors concluded that the true muscular tissue decreases in volume with advancing age and that the loss is replaced by adipose tissue. But the significance of their results is restricted firstly by the limitations of sampling as indicated by the great variation of chemically estimated fat in different parts of the gastrocnemius and in different muscles, and secondly by the nature of the necropsy material, which included many bedridden cases with debilitating illnesses. As these authors point out, prolonged severe disease was often associated with a remarkable accumulation of intramuscular fat and therefore these changes may well be related, at least partly to the nutritional state, the presence or absence of disuse atrophy the degree of cachexia of the selected subjects, or to a combination of all these factors. Inactive muscles often show a greater tendency to fat deposition than active ones. Moreover genetic, as well as nutritional influences need to be taken into consideration

V AGING CHANGES AND EXPERIMENTAL HORMONAL CONTROL

The relationship of endocrine glands to muscle is reviewed elsewhere in this book, but brief reference will be made here to the work of Korenchevsky and his co-workers (1950 1953), who have studied aging changes in the rat. They compared changes in cardiac and skeletal musculature, and many other organs, with changes following castration and the administration of pituitary thyroid androgenic, and ovarian hormones. In particular castration of male rats led to a degree of myocardial atrophy similar to that found in the untreated aging animal but these authors remark that the compensatory hypertrophy

T ...ıme 1956 for a review of these)

F... IN RELATION TO PATHOLOGICAL PROCESSES IN MAN; CONCLUSIONS

...s has been laid on the argument that in man, any ...es of muscle are often complicated or rendered ...e pathological lesions brought on by diseases to ...xposed and by secondary functional alterations. ...ant aging change in cardiac muscle consists in an ...n pigment the entity of simple senile atrophy is met ...when compared to the incidence of myocardial ...pertensive origin and of focal fibrosis due to ischaemic ... On the other hand a "brown atrophy" is often the expression of toxic changes in wasting conditions. Recently attention has also been drawn to a clinically silent form of primary atypical amyloidosis restricted largely to the myocardium and, in many cases, of microscopic distribution only which may not infrequently be met in subjects over 70 (Hüsselmann 1955 Lee and Kaufmann, 1957) The causes of this curious change, which has also been observed in senile mice (Thung 1957b) are as yet little understood although in mice genetic and dietary influences are known to play a part. The relationship of this form of amyloidosis to the aging process in comparative pathology has lately been reviewed by Thung (1957a)

In skeletal muscle, simple aging changes are even more difficult to distinguish from those referable to disease processes. It seems, however established that external ocular muscles, as well as a few other small muscles undergo from a relatively early age progressive alterations which are fairly characteristic and probably constitute a pure aging effect. Skeletal muscles elsewhere, on the other hand, preserve their

structural integrity practically throughout life and morphological changes are not expected to become noticeable in them until extreme old age even then, they exhibit a good deal of individual variation, which renders their evaluation uncertain Of greater importance in old age are the secondary changes in the skeletal musculature due to associated diseases and functional disturbances since morphology and chemical structure will naturally reflect the effects of wasting illness nutritional deficiency disuse, and immobility Atrophy may be the secondary manifestation of diseases of the joints and the nervous system, or changes in water and electrolyte content may be attributable to cardiac or renal impairment. In addition to these maladies commonly met in the senium, the latter may occasionally mark the onset of rarer more specific muscle disorders, like the variety of muscular dystrophy of the postclimacteric period described by Shy and Mc Eachern (1951) Wohlfart (1957) has also remarked that a non specific disseminated neurogenic atrophy is sometimes found in the elderly· the resemblances, already referred to between denervation atrophy and senile changes in the experimental animal raise the possibility that "aging changes" in muscles may in some cases solely reflect in the innervated fiber a nonspecific degeneration of its motor nerve supply Finally after changes have been assessed in the light of all these possible associations there remains a number of factors like the state of development and training of the musculature, and hormonal and probably genetic influences as well, which appear to play a large though indeterminate part in modifying the response in man of skeletal muscle to aging

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CHAPTER VIII

Post Mortem Changes in Muscle

J R. BENDALL

I INTRODUCTION

The most obvious change in the physical characteristics of muscles after death is the stiffening which sets in more or less rapidly and which is generally accompanied by the production of lactic acid. It is with this physical change and the underlying chemical changes that we shall be mainly concerned here.

The physical change of state from the highly extensible plastic condition of freshly excised muscle to the inextensible and rigid condition of muscle in full rigor has been noted from the earliest times, particularly in the field of forensic medicine, where empirical observation of the relation between the time of onset of stiffening and the physical condition of the corpse have been made with considerable precision. Thus, in early works in this field such as Taylor's (1844 1891) it was already noted that rigor set in earlier in the corpses of exhausted subjects such as soldiers killed in battle than in those of subjects who had died quietly in bed and this observation was correlated with the onset of putrefaction which was shown to be more rapid in the former case

than in the latter We know now that both these changes are dependent on the glycogen reserve of the muscle immediately after death which acts on one hand as a potential source of energy to keep the muscle out of rigor and on the other as a source of lactic acid which is itself a bacteriostatic agent. Thus, exhausted muscles, containing little or no glycogen, will pass more quickly into rigor than rested muscles and will finally attain a less acid reaction, allowing putrefaction to set in more rapidly

Thus, so far as description of the rigor process is concerned we cannot do better than turn to the early works of forensic medicine. On the other hand, the underlying chemical changes were considerably misunderstood until quite recently Even as late as 1933 (Starling 1933) it could be stated that rigor was due to the coagulation of the muscle proteins by lactic acid. This same theory is still repeated in the first edition of Bell and associates textbook (1950) The theory appears to have originated with Kühne (1864) and Schipiloff (1882) who studied the effects of acid on the water-soluble proteins of the sarcoplasm. *They were thus erroneously led to believe that changes in these constituents could determine the over all change within the intact muscle,* in spite of the fact that the major part of the muscle substance consists of the water insoluble contractile protein actomyosin. Moreover this theory ran counter to empirical observation, since it implied that stiffening should be directly dependent on the lactic acid produced, whereas this was known not to be the case. In fact, Claude Bernard (1877) had already shown that the so-called "alkaline" rigor in exhausted animals came on far more rapidly than acid" rigor, and his observations were repeated and extended by Hoet and Marks in 1926 The latter having thus cast doubt upon the lactic acid theory were led to postulate the existence of a third change, common to both acid and alkaline rigor and suggested that this might be the disappearance of one or other of the sugar phosphates then recently discovered. It was not, however until the work of Engelhardt and Ljubimova (1939) Erdös (1943) and Szent-Györgyi (1945) that the fundamental importance of ATP[1] to the physical state of muscle was fully realized. Erdös first showed that the

[1] The following abbreviations are used throughout
ATP ADP AMP adenosine tri- di- and monophosphates
ITP IDP IMP inosine tri- di- and monophosphates
P-C phosphocreatine
P inorganic phosphate
P_{al} acid-labile phosphate

onset of stiffening appeared to be correlated with disappearance of ATP from the muscle. He suggested that stiffening was due to the formation of rigid chains of actomyosin from the components actin and myosin, which are prevented from combining in the fresh muscle by the presence of ATP Erdös's observations were more fully investigated by Bate-Smith and Bendall (1947) and extended to include "alkaline" rigor It was shown that the time of onset of rigor varied directly with the initial glycogen reserve, that is with the potential production of lactic acid through the glycolytic cycle of Embden and Meyerhof (cf. Needham, 1942) Since this cycle resynthesizes ATP and maintains it at a high level, the connection between the two sets of findings is obvious. The duration of the rigor process, it now appears depends on three things the initial level of ATP the initial glycogen reserve, and the initial reserve of phosphocreatine (P C) which acts as an important source of resynthesis of ATP (Lohmann, 1935 Bendall 1951) Given a knowledge of these three quantities, the time of onset can be pre dicted within narrow limits. Even without this data, a reasonable prediction can be made merely by measurement of pH which reflects the stage to which the process has come (Marsh, 1954)

Another feature of the rigor process, implicit from its chemi cal basis, is its dependence on temperature. This, again has been completely misunderstood not only in popular works of fiction, but also in more serious studies (cf Taylor 1910), where it is often supposed that cold accelerates and heat slows down the rigor process Clearly such a supposition is against all chemical laws, and, moreover contra dicts the facts of experiment, which show that rigor is accelerated markedly by rising temperature, particularly in the range of 25 to 37 C The process differs, however from simple chemical reactions in that the temperature coefficient itself is not constant, but rises slowly in the range 0 to 25 C. and then ever more rapidly from 26 to 40° C (Marsh 1954) In general, therefore rigor will set in extremely rapidly under tropical conditions and much more slowly under the more temperate conditions in Europe

Side by side with the onset of stiffening the muscle gradually loses its "irritability" that is, its contractile response to electrical stimula tion In the old literature of forensic medicine, this loss was used as a test for the so-called death of the muscle, a point of no return, as it were. We find many extraordinary accounts of such observations. For instance Taylor (1910) reports the case of a decapitated corpse which

maintained its irritability for 20 hours after death, whereas the normal time for the completion of rigor does not usually exceed 12 hours, long before which the irritability of the muscle would have been lost. In fact, irritability in this sense seems to depend mainly on two factors, first the ATP content of the muscle, from which the energy for contraction is derived, and secondly the acidity of the muscle (pH) Thus, *although a muscle may contain ample ATP for contraction it will not* contract if the pH is too low The lower limit of pH is in the neighborhood of 6.5 (Marsh, 1952a Bendall, 1957) a point which is reached in normal experimental animals and probably also in the normal man some 6 hours after death (at room temperature) This, in fact, is about the time which most authorities agree is the limit for persistence of irritability in the human cadaver (Taylor 1910)

Once the rigor process has been completed, the muscle will remain rigid and inextensible for very long periods if it is kept free from bacterial contamination (Marsh, 1954), and no "resolution" of rigor will occur On the other hand if sufficient bacteria are present, more or less rapid putrefactive changes will set in, which will "plasticize" the muscle and enable it to be stretched quite easily although it cannot subsequently recover from the deformation (Taylor 1910) It is obvious that, in a rigid system of this type, relatively few chemical bindings need to be destroyed to render it easily deformable. During this phase, too certain other chemical processes which are not directly related to stiffening are also completed, such as the deamination of the adenine nucleotides to inosine nucleotides, *with liberation of ammonia* and the dephosphorylation and hydrolysis of the latter to free inosine and hypoxanthine.

II. PHYSICAL CHANGES

A. Extensibility

Of the physical changes which take place in the muscle after death, *the most easily measurable is the loss of extensibility to an applied* load. This is so because only one parameter is involved, the stretch deformation of the fibers. This method is to be preferred to other methods, such as penetrometer or sclerometer *measurements (Mangold* 1927) which are far more complex and difficult to interpret. In what follows, therefore, extensibility changes will be taken as the main criteria of the onset of stiffening

1 *Methods of Measurement*

The simplest form of extensibility measurement consists in setting up a strip of muscle in a conventional kymograph and applying a load to it by hand, and this method was adopted in early studies by Bate-Smith (1939) The stretch-deformation/time curve is long-drawn-out and continuous but either pre- or post rigor can be analyzed in terms of three com ponents, rapid moderately rapid, and slow These can be expressed in terms of three exponential curves, each with its own constant. Thus deformation in time, $t, = A(1-e^{-k_1 t}) + B(1-e^{-k_2 t}) + C(1-e^{-k_3 t}$

The effect of stiffening is then to reduce the over-all deformation in given time to 1/20th to 1/40th of the pre-rigor value. Analysis of th curves revealed that changes in components A and B were virtuall completed 6 min. after application of the load. By continuing th measurements for a further 2 min the contribution of component during the preceding time could be eliminated It was found also th the resting pre-rigor muscle gave a recovery curve on release from th load nearly identical with the preceding stretch curve, so that th ratio $A/(A+B)$ was virtually the same for both curves. On the oth hand if shortening occurred during the course of rigor this ratio fe considerably particularly for the recovery curve, but rose again to t pre rigor level as stiffening became completed

This method as outlined is clearly satisfactory for obtaining data c the detailed nature of the deformation curves but it is unnecessari cumbersome if a large number of measurements of extensibility are t be made during the course of rigor For this reason, it was abondone in later work in favor of a more convenient routine procedure, emplo ing the mechanical loading device, described by Bate Smith ar Bendall (1948) This method consists essentially of applying ar removing the load on the muscle by means of an electrically operat arm, so arranged that the muscle is alternately loaded and unloade every 8 min. until rigor is complete. The type of diagram obtaine is illustrated in Fig 1 It will be noted that the onset of stiffening very clearly indicated in each case by the marked decrease of exte sibility of which the best measure is the deformation or recovery the first 15 sec. after application or removal of the load (Bate-Smi and Bendall, 1949) If longer times than this are taken, any shorte ing of the muscle during rigor will tend to be included in the mea urement and will give an erroneously large value for the extension, for example in diagrams b and d in Fig 1 where considerable shor

ening occurred, as indicated by the upward displacement of the curves.

For the purpose of drawing smooth curves of the progress of stiffening therefore, the 15 sec. deformation is measured by extrapolation on each individual stretch and recovery curve during the course of rigor and the results are plotted as percentage loss of extensibility against time. In most experiments reported, the actual load on the muscle was set to give a pre rigor extension corresponding to about 15% of the rest length. This load generally amounts to about 50 g./sq cm. of cross section

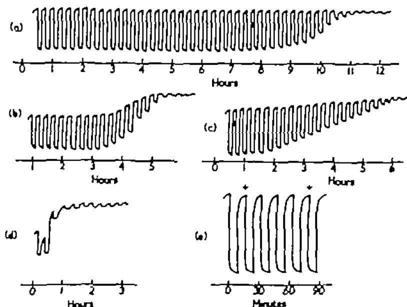


FIG. 1 Diagrams of extensibility changes during rigor in the psoas major muscle of the rabbit, recorded mechanically as described in the text. Muscles taken from (a) Well-fed rabbit, immobilized before death. Temperature 17°C., pH change: 7.00 to 5.70 during rigor (b) As (a) but muscle held at 37°C., pH change 7.05 to 5.80 (c) Well-fed rabbit, killed without prior immobilization. Temperature 17°C., pH change 6.52 to 5.72. (d) Animal exhausted by insulin convulsions at 17°C., pH 7.25 throughout. (e) Enlarged tracing of the early stage of rigor in a muscle such as (a) above ↓ means load on, ↑ means load off

The relation between load and extension is complex, but can be empirically expressed by the equation

$$\text{Extension} = k \log(\text{load}) - c \qquad (1)$$

where k and c are arbitrary constants.

When stiffening occurs, k diminishes twenty to fortyfold, but c remains more or less constant. This equation is almost identical with that of Hill (1952) relating tension to the fractional change of length of resting muscle.

2. *Decrease of Extensibility in Relation to the Length of the Muscle*

From the experience of work on the rabbit psoas (Bate-Smith and Bendall, 1956) the extent of the decrease of extensibility during rigor seems to be independent of any spontaneous shortening or lengthening which may have occurred, and also of the nature of the rigor whether "acid" or "alkaline." Thus in "acid" rigor at 17° C., occurring without shortening the extensibility decreased to ~ 1/38th of the pre rigor value, whereas in "acid" rigor at 37° C. accompanied by shortening of ~ 15% of the rest length, the extensibility decreased to ~ 1/33rd. Similarly in cases of "alkaline" rigor in which the shortening was ~ 19% of the rest length, the extensibility decreased to ~ 1/36th (Bendall, unpublished) Taking errors into account, these values do not differ significantly from one another in spite of the very different conditions in each case. On the other hand, Marsh (1953) has reported a series of experiments with beef muscle in which the extensibility decrease was found to be inversely related to the shortening which occurred The muscles which showed the most change, however were those held at 37° C. where there is a tendency for slight lengthening to set in on the completion of rigor (Marsh 1954), suggesting that the muscles may have slipped slightly in their attachments while shortening and have given erroneous extensibility values. The amount of "slip" required in this way to give a false idea of the extensibility would be less than 0.25% of the muscle length under normal loads of about 50 g./sq. cm.

3 *Time Course of the Changes*

The time course of the changes of extensibility are well illustrated by diagrams a, b c, and d of Fig 1 chosen from the large collection at the Low Temperature Research Station, Cambridge. The psoas muscle in diagram (a) was taken from a well fed rabbit, immobilized with myanesin for 20 min. before death, and was held thereafter at 17° C. It represents the maximum duration of rigor so far observed at this temperature. The diagram is seen to consist of three phases (1) a phase during which the extensibility of the muscle remains constant

and high duration about 11 hours. This phase will be called the delay period (2) In the next phase extensibility decreases rapidly but with very little change of length of the unloaded muscle. This is the rapid phase. (3) In the last phase extensibility has again become constant but at a lower level. This, the post rigor phase, lasts for many hours without change of extensibility until in fact, putrefaction sets in.

In contrast to this protracted rigor at room temperature, diagram (b) illustrates the effect of holding a similar muscle at 37° C. There is still a characteristic delay period with little or no change of the unloaded length, but it lasts for only about 4 hours against about 11 hours at 17° C. and is followed by a rapid phase during which the muscle shortens by about 15% of its rest length, as shown by the upward displacement of the curve. The extensibility falls to a minimum as the shortening is completed Diagrams (a) and (b) are characteristic of well fed and immobilized animals, or of animals which have died quietly On the other hand, if struggling occurs at death, as for example when a rabbit is killed by stunning and decapitation, the rigor diagrams of the muscles involved in the struggle, particularly the psoas, are more or less foreshortened and resemble the latter stages of diagram (a) An example of this type of rigor is given in diagram (c) where the extensibility decreases slowly at first and then ever more rapidly until rigor is complete. In such cases it is often difficult to distinguish the delay period clearly from the rapid phase, most probably due to the unequal rates of stiffening among individual fibers of the muscle. As we shall see later this type of rigor is characterized first by a low initial pH, indicating the production of much lactic acid during the struggle at death, and second by a much reduced initial level of P-C and ATP in contrast to quiescent muscles in which these three parameters are mutually very high. The duration of rigor however can be reduced even further than this by exhausting the animals before death by means of insulin or strychnine convulsions. Such cases are examples of the so-called "alkaline" rigor of Bernard (1877) where there is no production of lactic acid and no fall of pH which remains high throughout at about 7.2. An extreme example is given in diagram (d) where stiffening, accompanied by considerable shortening occurred almost immediately after excision of the muscle. In this case, there was no P-C present initially and the ATP level was already much reduced. Other cases of "alkaline" rigor have been described in which both these parameters were moderately high at death, with the

result that there was a delay period of about 90 min. before the rapid onset of shortening and stiffening (cf Bate-Smith and Bendall, 1947 1956)

Between the two extremes of "alkaline" and "acid" rigor represented by exhausted and well-fed animals, respectively lies the type characteristic of starved animals, that is animals in which the muscle glycogen has been more or less reduced. If such animals are immobilized at death, the duration of the delay period will decrease, but by no means so drastically as by allowing a struggle to occur For example, a 50% reduction of the initial glycogen content, that is of the potential glycolysis, results in curtailment of the delay period by about one third but in a somewhat prolonged rapid phase (cf Bate-Smith and Bendall 1949)

We can thus distinguish the following types of rigor

(1) Acid rigor characterized in immobilized animals by a long delay period and a fast "rapid phase," and in struggling animals by drastic curtailment of the delay period Stiffening is accompanied by shortening at body temperature only

(2) Alkaline rigor characterized by very rapid onset of rigor and marked shortening even at room temperature.

(3) An intermediate type, characterized in starved animals by curtailment of the delay period, but not of the rapid phase. Some shortening occurs during rigor at room temperature.

As we shall see later the type of rigor which will ensue at any temperature is strictly determined by the magnitude of the initial P-C, ATP and glycogen contents, of which the initial and final pH values are an indirect measure (Marsh 1954)

Similar results to those reported here for rabbit muscle have been obtained for various beef muscles by Marsh (1954) and Howard and Lawrie (1956 1957) and for horse muscle by Lawrie (1953) These studies were mainly confined however to "acid" rigor because of the difficulties of controlling the animals response to experimental treatments. Nevertheless, Howard and Lawrie (1956) were able to exhaust a bullock sufficiently by insulin convulsions to give an "alkaline" rigor characterized by precipitate onset, as in the example shown in Fig 1d The main differences found by these workers were in the duration and not in the overall pattern of rigor Particularly noticeable were the differences in duration between individual muscles of the same species, of which the extreme example is heart muscle where rigor is completed very rapidly after excision. Many of these differences however can be attributed to differences in the initial levels of P-C and ATP

B Concomitant Changes in "Texture"

Side by side with the changes of extensibility which occur during rigor there is a marked change in the "texture" of the muscle, which is soft and sticky before rigor and later becomes hard and dry as stiffening sets in. *This dry texture may later change to a moister condition* often characterized by an actual exudation of fluid, the so-called "weep." The extent of the "weep" is mainly dependent on the pH and the temperature, and becomes noticeable only when the final pH is well below 6.0. It is increased greatly by raising the temperature to 37° C., where the muscle, as it shortens, may squeeze out of itself as much as 15% of its weight as "weep" (Bendall, unpublished observations). "Weep" is entirely absent in cases of starvation or exhaustion, where the final pH of the muscles is above pH 6.3 and it cannot be induced under these conditions at 37° C. in spite of the considerable shortening which occurs. (Bate-Smith and Bendall, 1956). It must be stressed that, weep or no weep, the extensibility remains low and constant at this stage.

The change during rigor from a soft and sticky texture to a dry and hard texture can be assessed in a semi-quantitative way by means of a sclerometer (Mangold 1927), the principle of which is to measure the depth of indentation into the muscle substance of a weighted steel ball or plunger. *Clearly this method involves several parameters at once.* The actual hardness and extensibility of the muscle bundles, the ease with which they may be pushed apart, and the indeterminate changes in form of the irregular solid mass are concerned in the measurement recorded. In spite of this, the pattern of change closely parallels the extensibility changes, so that a "delay period," during which the "hardness" remains low but constant for several hours, is followed by a phase of rapid increase in hardness, which may in its turn be succeeded by a slower but considerable, decrease. This latter phase is particularly noticeable in whale muscle (Marsh, 1952a), where it is accompanied by a low pH (5.3) and the extrusion of much weep, probably due to severe damage to the sarcolemma, without which the loss of fluid would not be likely to occur. This would have the effect, from the point of view of sclerometer measurements, of converting the muscle bundles from firm "rods" of intact fibers to flaccid tubes of easily expressible fluid.

We can summarize the sclerometer findings tentatively as follows

(1) In the pre-rigor period, the low value for hardness is attributable to the low resistance of the intact and extensible fibers to sideways deformation by the steel ball.

(2) The increase in hardness during the onset of rigor is due to the sudden decrease in extensibility of the fibers, and their increased resistance to lateral deformation. At this stage, the sarcolemma is still intact, so that any fluid which may have been lost from the substance of the fibrils will be held within the fiber

(3) The observed decrease in hardness which may follow if damage is done to the sarcolemma under highly acid conditions is due to the ease of extrusion of fluid from the fibers and bundles of fibers, similar to the effect of pressure on a leaky tube of fluid. This has little or nothing to do with the extensibility of the fibers, which remains low and constant within the leaky membrane.

C. Is Rigor a Slow but Irreversible Contraction?

The idea that rigor is really a slow but irreversible contraction or contracture is mentioned frequently in the older literature (e g Taylor 1910) and even in more recent times (Bendall, 1951) it has been suggested that shortening is an essential part of the process. Considerable shortening can indeed occur during rigor either under alkaline conditions (e.g Fig 1d) or at higher temperatures (Fig 1b) and coincides in time rather exactly with the disappearance of ATP from the muscle. Stress has been laid on this feature as confirmatory evidence of the theory that it is the splitting of ATP which is responsible for contraction (Weber 1952 Bendall, 1951) but more recent studies of the shortening of muscle models in the presence of ATP have shown that by comparison, the shortening during rigor is feeble and yields only a tiny fraction of the power developed by the models and can be overcome with comparatively light loads (Bendall, unpublished observations) Evidence from histological studies also goes to show that the shortening in rigor never involves more than a fraction of the fibers, as can be seen from the photographs in Plate I which show (a) a longitudinal section of a muscle which went into rigor without shortening and (b) a similar section of a muscle allowed to shorten at 37° C. by about 40% of its rest length. In (a) all the fibers are straight and show marked cross striations, whereas in (b) a few fibers are straight, but without striations, and others are violently distorted. It is reasonable to assume that the straight fibers were the actively contracting ones, and

that in shortening they had caused the fibers on either side of them to fold passively into more or less irregular S-shapes. Although the difference between the two types of fiber cannot be attributed directly to any one chemical change, it appears likely that the active fibers are



PLATE Ia. Longitudinal sections of psoas muscles which went into rigor at 17°C without shortening

those in which ATP has been reduced rapidly to a level at which the relaxing factor of Marsh (1952a) can no longer exercise control over the contractile system. This hypothesis will be discussed more fully later

Similar arguments apply to rigor in ox and horse muscle, where

shortening may be considerable, but again must not be taken as an ability to perform large amounts of work. For instance, little or no shortening takes place if the load is increased above about 150 g./sq cm., whereas in the case of contraction in the living muscle, the op-



PLATE Ib. Longitudinal sections of psoas muscles which went into rigor at 37°C. with shortening of ~ 40% of the rest length.

timal load for the maximal work or power output is in the neighborhood of 1000 g./sq cm (Hill, 1939)

To summarize these findings we can say that "contraction" during rigor is always weak and slow that it extends over a period of hours instead of seconds and that, once complete, it is quite irreversible.

From the evidence of histological studies, a minority of the fibers have actively contracted whereas the majority have been merely passively compressed and distorted as the process continued. Rigor ensues in the shortened fibers, so that they become fixed at this shortened length. In muscles so shortened, it is these erstwhile "active" fibers which now entirely bear the applied load such muscles might appear to be more extensible, under heavy loads, than those which have not suffered shortening (cf. Marsh, 1953)

D The "Resolution" of Rigor

We have already mentioned in passing the so-called resolution of rigor but it is necessary to extend these remarks in an attempt to clear up the misconceptions which have arisen about it. It now appears certain that rigor measured in terms of extensibility does not pass off for many days under aseptic, laboratory conditions. Thus, beef muscles held at 7° C. in nitrogen for 7 days after development of full rigor showed no increase in extensibility (Marsh, 1954), and even at 37° C., no change of extensibility occurred in the 24 hours following the completion of rigor Similar results have been reported for rabbit muscle kept at 3° C. (Bate-Smith and Bendall, 1956) In all these experiments, no bacterial putrefaction was in evidence It thus appears that "resolution" of rigor is impossible without the intervention of some extrinsic agent or applied force, and from the abundant evidence of forensic studies, it is clear that putrefaction is the chief extrinsic cause (Taylor 1928) In spite of this evidence, however many physiologists have tried to show that it is due to some intrinsic change such as dissolution of myosin in the lactic acid produced (Hermann cited by Taylor 1928) coagulation of the proteins resulting in loss of hydrophilic properties (von Fürth, see Taylor 1928) or the release of destructive enzymes within the dead muscle. None of these suggestions appears to have any foundation in fact. It is sufficient to quote the oft repeated observation that the quicker the onset of rigor the quicker its resolution in the intact corpse. Since quicker onset is generally accompanied by production of less acid it is clear that the rate of resolution is inversely and not directly related to the acidity On the other hand, the more alkaline the conditions, the more rapid is the bacterial growth, so that it appears unnecessary to search for any other cause for the accompanying resolution than the proliferation of putrefactive organisms within the muscle tissue.

In this connection, the work of Barnes and Ingram (1955) is of interest, since they were able to show that the relative rate of anaerobic bacterial growth increased rapidly just after the onset of rigor what ever the acidity of the muscle This they attributed to a fall of the redox potential which reached a low value during this decisive phase, at least under the special conditions of their study Whether or not this fall of redox potential is common to both "acid" and "alkaline" rigor cannot be decided from their experiments. There is however another possible interpretation of the results. The greatest chemical change, other than acidification, which occurs under all conditions at the time of onset, is the conversion of adenine to inosine nucleotides. Since it is known that the former are far more toxic than the latter at least in animal tissues, this alone may account for the increased growth of bacteria.

E. The Structural Basis of the Physical Change

Before the advent of the electron microscope, many models were put forward to explain the stretch-deformation curves of muscle as measured before and after rigor Most of these originated from theoretical considerations involving a series of damped and undamped springs and were not founded on any real structures within the muscle itself apart from the well-recognized sarcolemma on the outside of the fiber and the contractile elements within. (cf. Weber 1934 Houwink, 1937 Bate-Smith, 1939) Hill (1939) had already criticized theories based on this conception of free and damped springs. He suggested on the other hand, that the main elements involved were a parallel elastic component, which took most of the strain when a resting muscle was stretched and a series elastic component, which came into play only in the contracting muscle He considered that the contractile elements themselves were too easily extensible under resting conditions to account for the deformation curves, which he attributed solely to the properties of the parallel component, perhaps identifiable with the sarcolemma. If this idea is considered in relation to rigor it is clear that the main change during the process must be in the contractile elements themselves since none of the concomitant chemical changes are likely to affect the extensibility of the sarcolemma significantly This of course has become even more likely since the discovery of actin as an essential part of the contractile mechanism and of the ability of this protein to combine with myosin in the absence of ATP

(Szent-Györgyi 1945) and by the further significant discovery that myosin is located exclusively in the A bands of the muscle (Hasselbach, 1953 Hanson and Huxley 1953 1955 1957) Nevertheless, a simple arrangement of parallel actin and myosin filaments will not account for the changes during rigor For this reason alone, the first muscle model proposed by Hasselbach appears to be inadequate This model consists of the schematic arrangement shown in Fig 2.

The actin filaments are taken to run in an unbroken chain through the I and A bands from one Z membrane to the next, and the myosin is located exclusively in the A band. Taken as it stands, this model

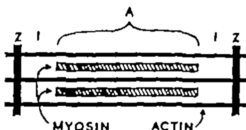


Fig 2 Muscle model of Hasselbach.

presupposes a high degree of extensibility to reside in the actin chains before rigor This extensibility is reduced during rigor by the formation of actin-myosin linkages. Unless further assumptions are made, the extensibility can be reduced by this means only by an amount proportional to the ratio of the I band length to A band length Since the I band at rest length is about half as long as the A band (Hanson and Huxley 1957) the total effect of reducing the extensibility of the material in the A band to zero would be to decrease the overall extensibility of the muscle threefold. In fact, the extensibility of a muscle at rest length decreases twenty to fortyfold during rigor as we have seen above. Thus, the simple model is at variance with the facts, unless a decrease in the extensibility of the actin chains of the *I band is also involved. It is difficult to see why this should come about.*

The model of Hanson and Huxley (1955 1957) on the other hand, explains the changes during rigor much more readily without involving any additional assumptions. This resembles Hasselbach s model in that the main elements are parallel and inextensible chains of actin and myosin. It differs, however in the important respect that the actin chains are considered to be discontinuous in the region of the H zone

of the A band and to be joined together by a protein of low density and high extensibility The myosin chains, on the other hand run right through this H zone. The Hanson and Huxley model is shown in Fig 3

This model behaves correctly so far as the pre-rigor muscle is con cerned where stretch deformation results in pulling the actin filaments out of the surrounding myosin with consequent lengthening of the H and I bands. The main deformation occurs in the highly extensible H band filaments. In the intact muscle, as opposed to the model, it is, of course, likely that the main stress is taken not by the H band

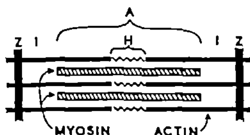


Fig 3 Muscle model of Hanson and Huxley

material but rather by the sarcolemma and connective tissue sheaths around the fibers and fiber bundles, respectively On the other hand, when the muscle goes into rigor the actin in the A band becomes bound to the myosin and the extensibility falls to that of the actin filaments, which is assumed to be low and constant throughout, and of the newly formed actomyosin filaments of the A band. Thus, the model competently explains the two most characteristic features of rigor the very great loss of extensibility and the independence of this extensi bility of the length of the muscle at the time

III. CHEMICAL CHANGES UNDERLYING THE PHYSICAL CHANGE

A. Changes Directly Related to Stiffening

The chemical change most easily measured during the course of rigor mortis in normal animals is the gradual acidification of the muscle, due to the production of lactic acid from glycogen through the glycolytic cycle of Embden and Meyerhof (*cf. Needham, 1942*) This change sets in at the moment of death, unless the oxygen supply to the muscle can be artificially maintained in some way or another

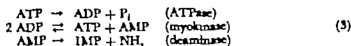
If the latter condition can be satisfied, as for example in thin strips in pure oxygen, it is possible to keep the muscle in an extensible pre rigor state for at least 48 hours after death, and then by changing to an atmosphere of nitrogen to bring on the normal acidification, and with it the onset of stiffening (Bendall, unpublished results) At first sight, this evidence suggests, as we have already had occasion to mention, that acid production is itself the immediate cause of stiffening and for a long time this was accepted as the case, although the work of Claude Bernard (1877) and of Hoet and Marks (1926) on "alkaline" rigor should have sufficed to show that this was not a general rule, and that some other change must be sought. This change was finally associated by Erdös (1943) with the loss of ATP from the muscle. This identification was made possible by the series of discoveries beginning with that of the ATPase activity of myosin by Engelhardt and Ljubimova (1939) and culminating in the demonstration by Szent-Györgyi and Straub (Szent-Györgyi, 1945) of the contractility of artificially prepared actomyosin threads in the presence of ATP Even so, the work of Erdös was not absolutely conclusive, because lactic acid production also occurred under the conditions of his experiments, whereas the general validity of the ATP hypothesis depended on showing the association of stiffening with the disappearance of ATP under all conditions, and particularly under conditions of alkaline rigor This was finally proved by Bate-Smith and Bendall (1947) Once these facts were established, it was possible to study the influence of other factors on delaying the loss of ATP and extending thereby the duration of rigor (Bate-Smith and Bendall 1949 Bendall, 1951 Lawrie, 1953 Marsh, 1954 Bendall and Davey 1957) As would be expected the chief factor is the mechanism for the resynthesis of ATP that is the supply of phosphocreatine (P-C) and of glycogen in the muscle which operates first through the Lohmann reaction



$$ADP + PC \rightleftharpoons ATP + \text{creatine} \qquad (2)$$

and secondly through the Embden Meyerhof glycolytic cycle, where three moles of ATP are resynthesized for every glucose unit of glycogen disappearing (Needham, 1942) This of course, presupposes that the determining step in the process is the splitting of ATP by one or other of the ATPases present in the muscle, since witho this primary event no resynthesis, that is, no l[illegible] or gl[illegible] d occur The resynthetic mechanism can[illegible] high and con

stant level as long as the P C supply lasts, so that at this stage the splitting and resynthesis of ATP are exactly in balance. Beyond this point, however splitting increasingly overtakes resynthesis, and the ATP level begins to fall, whether glycolysis is proceeding or not (Bendall, 1951 Lawrie, 1953) Simultaneously with this breakdown, ammonia and inosine monophosphate (IMP) appear in amounts equivalent to the loss of ATP probably through the chain of reactions



$$\begin{aligned} ATP &\rightarrow ADP + P_i \quad &(\text{ATPase}) \\ 2\,ADP &\rightleftarrows ATP + AMP \quad &(\text{myokinase}) \\ AMP &\rightarrow IMP + NH_3 \quad &(\text{deaminase}) \end{aligned} \qquad (3)$$

These reactions result in the rapid failure of the adenine nucleotide supply and the precipitate onset of rigor (Webster 1953a Bendall and Davey 1957) As Bendall and Davey have shown (1957) the ammonia liberated or the inosine nucleotide formed are nearly exact measures of the amount of ATP dephosphorylated, and are to be preferred for this purpose to the estimation of the acid labile phosphate ($P_{.}$) which was used as the criterion of the ATP level in earlier work (cf. Bate Smith and Bendall 1956) The latter parameter can be shown by detailed chromatographic analysis to be reliable only in the early phase of rigor when the ATP level is high and constant and little or no IMP has yet been formed Later however, acid labile phosphate esters of non-nucleotide origin are formed in amounts which may seriously invalidate the P values as a true measure of the ATP level For this reason, the results reported here are calculated on the assumptions (a) that the initial P_{t} values represent the true initial ATP level and (b) that the subsequent formation of inosine nucleotides, measured spectrophotometrically exactly represents the loss of ATP The latter assumption is not quite justified because, as we shall see, trace amounts of ITP and IDP besides IMP are formed during rigor This introduces a positive error of not more than 2% of the total nucleotide content. Apart, however from the rather cumbersome, but more accurate chromatographic methods, the scheme outlined represents the quickest and most reliable way of estimating the ATP level. It will be noted from Fig 4 that it removes the apparent anomaly frequently encountered in earlier work by Bate-Smith and Bendall (1947 1956) of the persistence of large amounts of labile P_i in the post rigor period, which were evidently of non-nucleotide origin.

The time course of the chemical changes during acid rigor is best illustrated in the muscles from well-fed animals, immobilized before

death. These conditions ensure high initial levels of glycogen, P-C, and ATP and also a high initial pH, so that the duration of rigor and the production of lactic acid are both maximal (Fig 4) The production of lactic acid (LA) and loss of glycogen (G) are given in these experiments in terms of the fall of pH to which they are linearly related as shown in Fig 5 It will be noted that, in the case of glycogen,

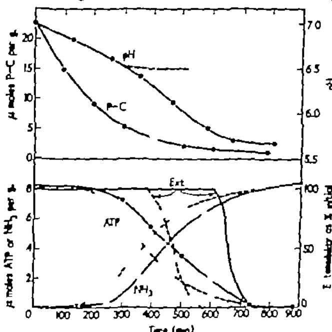


FIG. 4 Time course of the chemical and physical changes during rigor in the psoas muscle of an immobilized animal. Temperature 17°C.
Full lines —— well-fed animal. pH change 7.0 to 5.6.
Broken lines – starved animal. pH change 7.0 to 6.5.

this linear relation holds only from pH 7 1 down to about pH 5.80 at which point glycolysis slows down, so that varying amounts of glycogen are left in the muscle after the changes are complete. This is the so-called "residual glycogen" described by Lawrie (1955) in horse muscle. The pH at which this residue accumulates appears to vary considerably in different muscles of the same species. Taking this proviso into account, we can write for the rabbit psoas

$$\frac{\Delta LA}{\Delta pH} = \frac{\Delta G}{\Delta pH} = 63 \ \mu eq \ H/pH/g \text{ of muscle (from pH 7.2 to 5.8)} \equiv \text{resynthesis of } 97.5\,\mu \text{ moles of ATP from ADP}^{a}$$

[a] See Bate-Smith and Bendall (1956)

The most characteristic features of the early stages of chemical change (Fig 4) are (a) the low rate of fall of pH, that is rate of glycolysis, and (b) the steady level of ATP Both of these factors remain constant until the P C level has fallen below ~ 5 μmoles/g Beyond that point, however the rate of glycolysis accelerates to about 1½ times the initial rate and the ATP level begins to fall accompa

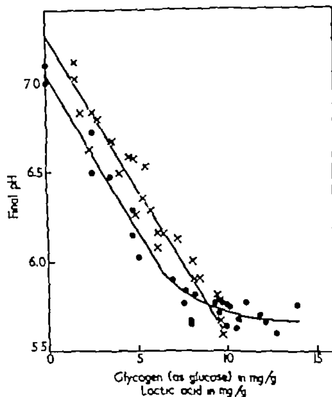


FIG. 5. Plot of initial content of glycogen and of final content of lactic acid against final pH of the muscle. Animals immobilized before death. • = Glycogen, × = Lactic acid.

nied by the production of ammonia in equivalent amounts [see Eq (3)] This fast phase begins at pH 6.5 that is when ~ 3 mg of glycogen/g have been lost. This clearly illustrates the "sparing" effect of P C on ATP and glycolysis, although the increased rate of glycolysis which subsequently sets in more than compensates for the loss of resynthetic potential, as we shall see later

It will also be noted that the extensibility decrease does not begin until the ATP level has fallen to ~ 2 μmoles/g., the critical level and

then proceeds rapidly. This critical level of ATP, however, is not constant for all types of rigor, but varies with pH and is much higher in alkaline than in acid rigor.

The changes illustrated in the figure took place at 17° C., but their general pattern is the same whatever the temperature. Thus, at 37° C., the rate of all the changes is accelerated almost exactly 2.5 times, but the relation of the changes, including the extensibility change, to one another remains unchanged. Similarly, the changes at 0° C. are 1.3 times slower than at 17° C., but again without change of pattern (cf. Bendall and Davey, 1957).

The pattern of changes can be markedly altered at all temperatures either by starving or exhausting the animal, or by allowing struggling to occur at death. The effect of starvation is, of course, to reduce the initial glycogen content, so that the final pH in full rigor is generally above 6.5 instead of below 6.0, as in well-fed animals. This does not affect the rate of loss of P-C or of fall of pH, but affects the other parameters as shown by the dotted lines in Fig. 4. It is seen that the ATP level now drops somewhat earlier, although at nearly the same rate. In contrast to these relatively small differences in the pattern of chemical change is the marked curtailment of the delay before the extensibility begins to fall, although the fall, once begun, is slower than in the first case. In these cases, the critical level of ATP is now raised to $\sim$ 4 μmoles/g.

When struggling occurs at death, the muscles which are involved, such as the psoas, show a characteristically low initial pH, a high initial LA content, a low initial level of P-C, and a reduced level of ATP, which falls from the moment of death, accompanied by further acidification, the extent of which depends on the initial glycogen content. In fact, the pattern of change closely resembles the later stages of Fig. 4, characteristic of immobilized animals. There is the important difference, however, that neither the extensibility change, nor the disappearance of ATP, are well-defined, evidently due to varying rates within the individual fibers which took part in the death struggle.

Although struggling at death may hasten the onset of rigor by several hours, the effect of complete exhaustion of the animal before death, e.g. by means of insulin or strychnine convulsions, is even more pronounced and usually gives rise to precipitate onset as illustrated in Fig. 1d. In this case, which is an example of the so-called "alkaline rigor" of Bernard (1877), there was no glycogen in the psoas at death,

no LA production or fall of pH, no P-C, and a lowered level of ATP The extensibility was lost almost immediately on excision. This is typical of alkaline rigor in the psoas, although very occasionally examples are found where the initial P C and ATP contents are high and the onset of rigor is consequently delayed for about 80 min. after death, (cf. Bate-Smith and Bendall, 1956) Cases of the latter type illustrate the effect of P-C on the rates of change when glycolysis is entirely absent. Such cases show that, by itself P-C is no more effective than glycolysis alone in prolonging the delay before onset of rigor It is, in fact, only when both resynthesising mechanisms act together that rigor can be long delayed. The reason for this may be that ATP is not only attacked by ATPases, but is also lost during glycolysis, at the stage of the phosphorylation of hexose-6-phosphate through the reaction



$$ATP + hexose\text{-}6\text{-}P \rightarrow fructose\text{-}di\text{-}P + ADP \qquad (5)$$

If the resulting ADP can be rephosphorylated quickly enough, as is the case when P-C is available, there will be little chance of other enzymes, such as myokinase, attacking it (myokinase would produce a molecule each of ATP and AMP from two molecules of ADP) Thus, at this stage there is no drain on the ATP level. When the P-C level is low or zero however the myokinase reaction will convert some ADP to AMP in this way and the latter will be immediately deaminated to IMP by the active fibrillar deaminase, thereby irrevocably draining away the adenine nucleotide supply through equation (3) Thus, ATP always disappears rapidly if glycolysis is unsupported by the P-C-kinase reaction. On the other hand, when P-C is the only source of resynthesis, as in alkaline rigor it will bear the whole brunt of the attack by the ATPase, and will be drained away much more quickly than when supported by glycolysis.

We can summarize the chemical changes as follows

(1) Glycolysis, measured as fall of pH proceeds at a slow rate from the moment of death until the P-C level has been reduced to about 5 µ moles/g It then accelerates, and as this happens the ATP level begins to fall, with equivalent production of IMP and NH_3

(2) If glycolysis is restricted by starvation, the ATP level falls somewhat earlier than in the presence of adequate glycogen.

(3) If the P-C level is reduced by struggling at death, the ATP level falls almost immediately as it does when the glycogen content is abolished completely by extreme exhaustion.

(4) These findings illustrate (a) the sparing effect of P C on ATP and (b) that neither glycolysis nor P-C alone can delay the disappearance of ATP for long

(5) The ATP level at onset of stiffening varies with the pH at the time.

B THE EFFECT OF TEMPERATURE ON THE PHYSICAL AND CHEMICAL CHANGES

The effect of raising the temperature from 17 to 37° C. has been superficially discussed in earlier sections, when it was seen to consist of (a) a 2½ fold increase in the rates of change and (b) a marked increase

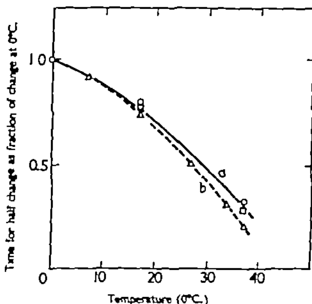


FIG. 6. Plot of temperature against (a) times for half change of ATP (⊘) P-C (x) and extensibility (□) in rabbit psoas, post mortem (b) time for fall of pH from 7.0 to 6.2 in beef muscle (Δ) (after Marsh, 1953) Times given as fractions of time at 0°C

in shortening during rigor The temperature effect is shown in more detail in Fig 6, where temperature is plotted against the times for half change of P-C, ATP and extensibility respectively as fractions of the time at 0° C. The three parameters decrease almost identically as the temperature is raised the rate of decrease in each case accelerating markedly above 25 C. This acceleration is even greater in beef muscle (dotted lines), with the result that the duration of rigor which is about

the same in both animals at 37° C. is 1½ times greater in beef than in rabbit muscle at room temperature (Marsh 1954)

The effect can be expressed in another way by considering the activation energy of the process, which is basically that of the rate-determining step the splitting of ATP. Thus, the activation energy in the range of 0 to 25° C. is $\sim 3 \times 10^3$ g cal. in beef and rabbit muscle, but rises abruptly above this point to a new level of $\sim 2 \times 10^4$ g cal. in beef and of $\sim 1.2 \times 10^4$ g cal. in rabbit. The reason for this abrupt increase is not clear but it could be taken to mean either that two enzymes are involved in the primary step or that one ATPase alone is

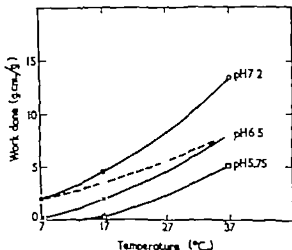


FIG. 7 Plot of temperature against work done during rigor in: (a) rabbit psoas muscles at three different levels of final pH (full lines) (b) beef muscles (broken line) at final pH values of ~ 5.70 (after Marsh, 1953)

responsible, the physical state of which changes critically with temperature. In this respect, the main ATPase of the muscle, that associated with the myofibrils, behaves quite differently and has a nearly constant activation energy of $\sim 2.5 \times 10^4$ g cal. when activated by Mg^{++} and of $\sim 1.2 \times 10^4$ g cal. when activated by Ca^{++} over the whole range from 0 to 35° C. (Bendall, unpublished observations)

Unlike the chemical changes, the degree of shortening and the amount of work done during rigor depend not only on temperature but also on pH (cf Bendall, 1951) The effect is, however greater above 25° C. than below as with the other changes (Fig 7) We can say therefore, that the effect of temperature on the chemical and ex

$(NH_4)_2SO_4$/100 ml. (Kumagai *et al.*, 1955) and later in the sarcoplasmic granules which are present in this fraction (Portzehl, 1957 Weber 1957) It has been shown to possess the following properties

(1) It inhibits the Mg activated myofibrillar ATPase completely at all concentrations of ATP (Marsh, 1952b Webster 1953a Bendall, 1953 Hasselbach and Weber 1953 Briggs and Portzehl, 1957) This inhibition is relieved by traces of Ca^{++} but not by Mg^{++} even in excess.

(2) Because it inhibits the ATPase, the factor reverses the ATP induced syneresis of myofibrils (Marsh, 1952b) and induces relaxation in loaded fiber models (Bendall 1953 Hasselbach and Weber 1953)

(3) It is sensitive to ionic strength, and is ineffective below $I = 0.05$.

These properties distinguish the factor from the over-optimal effect which is overcome by addition of excess Mg^{++} and is relatively insensitive to ionic strength (Perry 1956) This distinction is made plain by comparing the two effects under identical conditions, as in Table I (Bendall, unpublished observations)

TABLE I

EFFECT OF OVER-OPTIMAL (ATP) AND MARSH FACTOR ON THE MYOFIBRILLAR ATPASE ACTIVITY

(ATP) in mM	Splitting as μ atoms P_i/mg N in 2 min. Fibrils alone	Fibrils plus factor
2	2.8	0 40
4	3.0	0
6	2.1	0 06
8	1 4	0.30
10	1 1	0.30

Factor prepared by precipitation from muscle extract with 15g $(NH_4)_2SO_4$/100 ml, followed by dialysis. Buffer 5 mM phosphate/160 mM KCl pH 7.22, $MgCl_2$ = 4 mM concentration of myofibrils ≅ 4 mg. protein/ml of factor ≅ 3 mg. protein/ml. No ATPase activity in factor alone.

From this it is seen that the factor completely inhibits splitting at ATP concentrations at which the Perry effect is minimal. This argues strongly in favor of the factor as the natural mechanism controlling the myofibrillar ATPase activity It should be noted however, that it will inhibit only the myofibrillar ATPase and not the relatively feeble sarcoplasmic enzyme.

Although we have seen that the myofibrillar enzyme need show only about 1/100th of its maximal activity to explain the very low rate of

splitting during rigor there are the following reasons for believing that it does not participate even to this extent

We know that myofibrillar ATPase activity always results in development of tension or performance of work, since it is evidently from this source that contraction in vivo directly derives its energy (Weber 1956 Perry 1956) If the splitting during the delay period of rigor were due to this enzyme, we should expect it, therefore, to be accompanied by shortening and the performance of work, whereas no such change of length occurs at this time, but only later during the phase of rapid ATP-disappearance. Even under optimal conditions however the work done rarely exceeds 15 g cm./g Assuming an efficiency of 30% and a free energy change of 11 millical /μmole of ATP split to ADP this amount of work would require the splitting of no more than 0 1 μmoles of ATP, yet the actual amount of ATP split while the muscle is shortening is ~ 4 μmoles, equivalent to a potential work performance of ~ 600 g cm./g Thus, even during shortening the myofibrillar enzyme contributes < 3% to the total splitting the other 97%, being due to some other ATPase which is mechanically without effect.

From these arguments we may conclude that the myofibrillar enzyme plays no significant part in either the chemical or physical changes during rigor and that control over it by the Marsh factor is more or less complete.

The sarcoplasmic ATPase, associated with the granules (Kielley and Meyerhof, 1948 Perry 1952a) is the only other well-defined ATPase of muscle, and this alone suggests that it must be responsible for the splitting during rigor Its pH/activity curve differs, however from the characteristic curve of Fig 8 in having an optimum at pH 7 0 instead of 6.2. This does not necessarily argue against it, because it may be extremely sensitive to its environment as are other particulate ATPases, such as that of liver (Kielley and Kielley 1951) Enzyme activity of this type remains latent under mild conditions and is only "released" as the conditions become more severe (Harman and Kitiyakara, 1955) It may be, therefore that the pH change during rigor is a sufficient stimulus to release the activity so that the apparent pH activity curve is really a pH/latency curve and does not truly represent the activity of the free enzyme. Moreover a latency phenomenon of this type might well explain the confusion which exists over the so-called soluble ATPases of muscle, such as that reported by Salov (1941) to have a pH optimum at 6 0 and the similar enzyme

studied by Humphrey and Humphrey (1950) Still another point in line with these arguments is the abrupt increase in the activation energy above 25 C. (section III, B) which might be expected in the case of an enzyme of this sort, due to heat lability

We may conclude, therefore, that the main splitting during rigor is attributable to a sarcoplasmic enzyme, probably identical with the granular ATPase of Perry (1952a) It follows that the low ATP turnover characteristic of living resting muscle must also be due to this enzyme (Kalckar 1944 Bendall, 1951)

D The Critical Level of ATP at Onset of Rigor

We have seen earlier that the initial level of ATP at which rigor sets in is about 2 μmoles/g at low final pH values, that is in acid rigor and about 4 μmoles/g in alkaline rigor These differences in critical level have been attributed to the different rates of resynthesis in the two cases (Bate-Smith and Bendall, 1956 Bendall, 1951) suggesting that stiffening is dependent not so much on a critical level of ATP, as on a lowered level associated with a decay of resynthesis. Thus, in alkaline rigor the ATP level is often not "supported" by any resynthesis whatsoever whereas in acid rigor resynthesis from glycolysis continues until almost all the ATP has disappeared This hypothesis certainly accounts for the facts, but it now appears unnecessarily cumbersome in view of more recent experimental work. Thus, Briggs and Portzehl (1957) have shown that relaxation in the presence of Marsh factor requires a lower concentration of ATP at low pH values than at high, and a similar finding has been reported by Watanabe and Sleator (1957) in a study of relaxation in the presence of EDTA. These facts are the direct corollary of what is found in rigor so that it now appears that it is pH alone which determines the sensitivity of the actomyosin system to ATP Thus, if there is sufficient glycogen to lower the pH during the phase of rapid ATP disappearance, stiffening will set in at a lower ATP level than when the pH fall is restricted through lack of glycogen. The critical point is fixed in each case by the combined effect of pH and ATP level.

E. Other Chemical Changes

Besides the parameters which directly affect the time course of stiffening such as the resynthesizing potential and the level of ATP there are a number of other changes which occur simultaneously but which are completely unrelated to the physical change. Of these, the

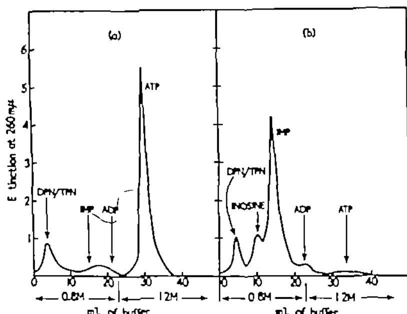


FIG. 9 Elution of nucleotide components of muscle from columns of Dowex 2 (14 × 0 9 cm.) by sodium monochloracetate buffers at pH 4.2 The volume of buffer is arbitrarily plotted against the extinction readings of the fractions at 260 mμ. (a) Pattern pre-rigor (b) Pattern post-rigor

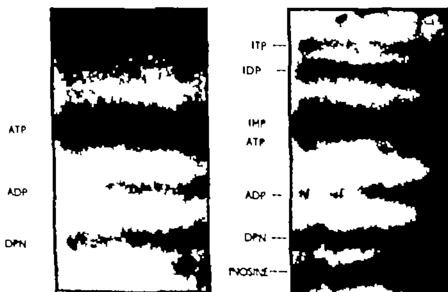
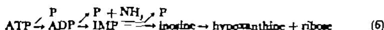


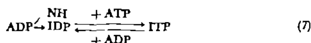
PLATE II Paper chromatographic analysis of the nucleotides of the rabbit psoas a pre-rigor (b post-rigor (Taken from Bendall and Davey 1957)

most striking are the changes in nucleotide pattern (Bendall and Davey 1957) As we have seen, the main change in the nucleotides consists in the disappearance of ATP and the formation of IMP from it by dephosphorylation and deamination, which can easily be demonstrated by ion-exchange chromatography (Fig 9) In the case illustrated, the dominant peak before rigor was ATP with much smaller ADP IMP and DPN/TPN peaks. After rigor the ATP peak disappeared and IMP and inosine appeared in equivalent amounts, whereas the DPN and ADP peaks remained more or less unchanged In some cases, the ATP peak does not disappear so completely These, however represent only the main changes, for similar samples examined by paper chromatography show the presence of at least seven bands after the completion of rigor (Plate II a and b) These consist of a dominant band of IMP with a smaller ATP band close to it, a DPN band unchanged in intensity and two bands above IMP which can be identified as ITP and IDP and one below DPN which can be identified as free inosine. In samples taken 48 hours after death, free hypoxanthine is also present (Davey 1957) showing that the following chain of reactions has occurred



$$\mathrm{ATP} \xrightarrow{\mathrm{P}} \mathrm{ADP} \xrightarrow{\mathrm{P} + \mathrm{NH}_3} \mathrm{IMP} \xrightarrow{\mathrm{P}} \text{inosine} \rightarrow \text{hypoxanthine} + \text{ribose} \qquad (6)$$

Simultaneously with this main breakdown, a subsidiary chain is evidently possible



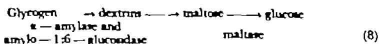
$$\mathrm{ADP} \xrightarrow{\mathrm{NH}} \mathrm{IDP} \underset{+\mathrm{ADP}}{\overset{+\mathrm{ATP}}{\rightleftharpoons}} \mathrm{ITP} \qquad (7)$$

The first reaction of equation (7) is known to be catalyzed by actomyosin (Webster 1953b Deutsch and Nilsson 1953) and the second by the so-called *nucleotide-diphosphate-phosphokinase* (Krebs and Hems, 1953) There are, however difficulties in accounting for the kinetics of the chain, because (a) the deamination of ADP is so slow that it would be thought unable to compete with the much more rapid myokinase and glycolytic reactions for the small amounts of ADP available, and because (b) ITP is split by the myofibrillar ATPase (Perry 1956) and is not protected from such attack by the Marsh factor (Bendall, 1954 Weber 1956) To overcome these contradictions, it has been suggested that it is the ADP bound on actin [Pettkó and Straub 1949 Perry (1952b) Hasselbach, 1957] which is slowly

deaminated as rigor is completed, (Bendall and Davey 1957) but this is satisfactory only if it is assumed that the rest of the subsidiary chain takes place close to the binding sites of the ADP in such a way that the products themselves become bound and so protected from attack by other enzymes. At present, the argument cannot be carried further except to draw the analogy with the phenomenon recently described by Hasselbach (1957), who found that the bound ADP of actin could be split by potato apyrase to AMP and P without release of products from the bound state.

Besides these changes in the nucleotide fractions, which are the culmination of the rigor process, other changes in chemical composition occur during and immediately after rigor One of these has already been mentioned the accumulation of P of non-nucleotide origin There seems little doubt that the main part of this fraction consists of hexose-diphosphate which arises at the stage of phosphorylation of hexose-6-phosphate by ATP (Sharp personal communication) This ester accumulates to a much greater extent during acid rigor (~ 4 μmoles/g) than alkaline rigor (~ 1 μmole/g) at room temperature or below and is present only in traces at the end of either type of rigor at 37° C. (Bendall and Davey 1957) From this, it is evident that some enzyme lower in the glycolytic cycle is partially inhibited as the pH falls

Another change which was frequently described in the older literature, but was neglected after the discovery of phosphorylative glycolysis, is the breakdown of glycogen to glucose through the reaction



This chain of reactions, studied in detail by Sharp (1957 and personal communication) seems to occur from the moment of death although only at about one-tenth the rate of the phosphorylative cycle it continues until glycogen has entirely disappeared Thus, it may introduce serious errors into the estimation of the "residual glycogen" of Lawrie (1955) unless samples are taken immediately on completion of rigor

We can summarize these miscellaneous changes as follows

1) The post rigor breakdown of nucleotides continues beyond the formation of IMP to give inosine and free hypoxanthine ITP and IDP are also formed in trace amounts at this stage.

2) Hexose-diphosphate accumulates during acid rigor at room

temperature or below to the extent of ~ 4 μmoles/g It is found only in traces in alkaline rigor or rigor at body temperature.

(3) Glycogen can be split to glucose by a non phosphorylative route the rate is about one-tenth of that of phosphorylative glycolysis.

IV ABNORMAL TYPES OF RIGOR

A. Thaw Rigor and Thaw Contracture

The phenomenon of thaw rigor that is the rigor which occurs on thawing out a frozen muscle, has been known for many years (Chambers and Hale, 1932) Thaw rigor has been described more recently in relation to ATP turnover by Perry (1950) in frog muscle and by Szent-Györgyi (1950) in rabbit muscle. It differs from normal rigor in that its time of onset depends only on the rate of thawing and that it is always characterized by a more or less powerful contracture, the extent of which is directly related to the ATP content (Perry 1950) Thus muscles frozen pre rigor develop more power on thawing than those which have been frozen with an already depleted ATP level, although limited thaw contracture can occur even at very low levels. For instance, the author has encountered a case of a muscle, frozen just after completion of normal rigor which shortened considerably on thawing despite an ATP content of only ~ 1.5 μmoles/g Another characteristic of this type of contracture is the accompanying extrusion of large amounts of sarcoplasmic fluid the so-called "drip" which may amount to 25% of the wet weight.

The phenomenon, besides its general biochemical interest, is of some concern in the commercial quick-freezing of meat. Trouble was encountered, for instance in the blast freezing of whale muscle, where the onset of rigor is often so long delayed that pre-rigor freezing becomes possible. Sharp (personal communication) has reported, for example that whale steaks, grilled from the frozen state, frequently curl up and contort themselves, with expression of much fluid, as thawing proceeds. Such examples always show a high pH in the frozen material i.e. a high ATP content (cf. Sharp 1953) On the other hand, blast freezing of meat "on the bone" rarely gives rise to noticeable thaw contracture, partly because only a limited depth of muscle can actually be frozen pre rigor as in the case of bullocks (Howard and Lawrie, 1956 1957) and partly because the rigid structure prevents shortening as in the case of lambs, where the whole

depth of muscle can be frozen in the pre-rigor state (Marsh, 1957)

The physical and chemical changes during thaw contracture will be described here, mainly from the author's unpublished observations on the rabbit psoas, because these seem to give a rather fuller picture than can be obtained anywhere in the published literature.

1 *Chemical and Physical Changes*

a The Contracture Phase As the rate of thaw contracture depends primarily on the rate of thawing it follows that maximum power output can only be expected in very thin fiber bundles thawing

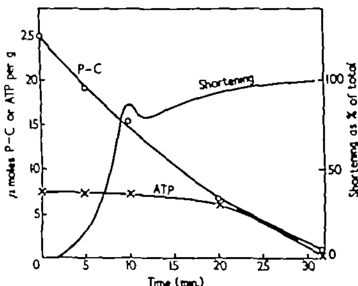


FIG. 10 Pattern of chemical and physical changes in a psoas muscle strip, frozen pre-rigor at −20°C. and subsequently allowed to thaw in air at 17°C. Approximate dimensions of the strip were 0.5 × 1.5 × 8 cm. Load = 67 g./sq cm. Total shortening = 58% of initial length. pH change 7.02 to 6.10

extremely rapidly These, however are too small for detailed chemical analysis, for which it is necessary to use much thicker strips with consequent loss of power Nevertheless, such strips show the distinctive features common to all thaw contractures, as we see from Fig 10 It will be noted first, that the pattern of decay of P C and ATP is very similar to that in normal rigor but unfolds in minutes instead of hours (cf. Fig 4) Second, the contracture begins almost immediately on

thawing, and is complete before the ATP level has decayed significantly, in contrast to normal rigor where shortening occurs only in the fast phase of decay (see Sections III C and IV C). Third, there is a characteristic notch in the shortening curve, which in thinner strips



PLATE III. Longitudinal section of a muscle, frozen at −20°C. and subsequently thawed in air at + 17°C., with shortening of ∼ 50% of its rest length.

develops into more or less complete relaxation (cf. Perry, 1950; Szent-Györgyi, 1950). Fourth, the power output, although not maximal, is far higher than in normal rigor: in this case, for example, power output was 4 g. cm./min./g., compared with the maximum in alkaline rigor at 37° C. of ∼ 0.5 g. cm./min./g. (cf. Bendall, 1951). Fifth, the minimal

ATP turnover calculated in the absence of accurate pH data, is ~ 2.5 μmoles P_i/min./g., which is ten times higher than the rate in normal rigor at this temperature (see Section IV C)

These facts taken together suggest immediately that the myofibrillar enzyme has been more or less activated by freezing and thawing There is, indeed, no other way of explaining the high power output and, particularly the increased ATPase activity which is nearly double the maximal possible rate of the sarcoplasmic enzyme esti-

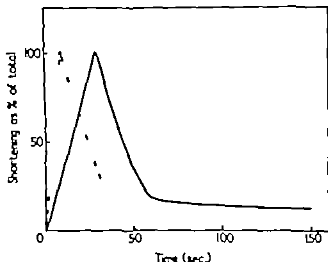


FIG. 11 Spontaneous relaxation (full line) on thawing of a thin strip of psoas muscle, previously frozen pre-rigor at −10 C., in liquid paraffin. Dimensions 0.12 × 0.12 × 2.6 cm. (Load = 84 g./sq cm. Total shortening 50% of initial length.) Broken line shows KCl-contracture of a phasic muscle (after Sandow 1955)

mated from the rate in isolated systems (Bendall, 1953 Webster 1953a) Nevertheless, the power output is still very low and represents only ~ 1% of that calculated from the splitting rate at an efficiency of 30%. As we shall see later this is probably due to spontaneous relaxation which, in thinner strips, sets in quickly after the initial contracture. This would have the effect of allowing the completion of contracture and the onset of relaxation in the rapidly thawing outer fibers of the thick strip while the core was still frozen, thus drastically reducing the power The much distorted histological picture shown in Plate III supports this conclusion.

Turning now to a very thin muscle bundle (Fig 11) frozen in

liquid paraffin at $-10°$ C. and thawed out 5 hours later at $+20°$ C., we see that contracture is almost instantaneous and develops much greater power than in thick strips. In this instance the power output was ~ 400 g. cm./min./g. which, in the author's experience, is maximal. The contracture lasted, however, for only 30 sec. and was succeeded by rapid relaxation. No further change of length occurred during the succeeding 10 min. Thus, if the thawing of the entire mass of a thin strip is sufficiently rapid, we obtain a picture very similar to the spontaneously reversible KCl-contracture of intact "phasic" muscles

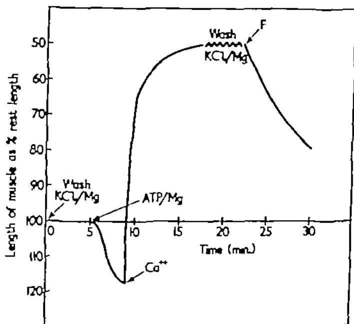


FIG. 12. Effect of ATP on a muscle bundle, frozen pre-rigor and subsequently allowed to thaw isometrically at rest length in isotonic KCl solution. Cross sectional area 0.09 mm. Load = 750 g./sq. cm., KCl/Mg = 160 mM KCl + 4 mM $MgCl_2$, ATP/Mg = KCl/Mg + 5 mM ATP. Ca^{++} = addition of $CaCl_2$ to 0.2 mM. F = crude Marsh factor + the above ATP solution.

(cf. broken line in Fig. 11, taken from Sandow, 1955). This suggests that in both cases it is the salt "flux" which swings over the very delicate balance on which the contractile machinery is poised, and that when this "flux" has had time to equilibrate, the conditions for relaxation are re-established in spite of depolarization of the muscle membrane and loss of membrane potential. Whether relaxation actual-

ly occurs or not will then depend solely on the availability of sufficient ATP

The implication of these findings is that the relaxing factor of Marsh, (1952b) is not destroyed, but only temporarily inactivated, by freezing and thawing. This can be proved conclusively by the experiment illustrated in Fig 12 Here a thin bundle of muscle fibers was frozen pre-rigor in air and allowed to thaw out isometrically in isotonic KCl solution at its rest length It was then released and a suitable load applied On addition of ATP KCl $MgCl_2$ solution, the bundle immediately lengthened under the load, showing the presence of relaxing factor This relaxation was instantaneously and characteristically overcome by addition of a trace of $CaCl_2$, and was succeeded by powerful shortening On washing out the Ca^{++} and replacing it by Marsh factor + ATP — $MgCl_2$ however the bundle once more relaxed. Thus we have a contraction relaxation cycle which can be artificially reproduced several times in the thawed muscle.

A possible interpretation of thaw contracture follows from these results

(1) The first effect of thawing is to bring about an extensive salt flux through the muscle membranes, which releases some of the Ca^{++} bound to the myofibril (Hasselbach, 1957) thereby inhibiting the relaxing factor stimulating the myofibrillar ATPase and thus setting the contractile machinery in motion.

(2) If thawing is almost instantaneous, however the salt balance throughout the mass of fibers quickly equilibrates, with the result that the small amount of Ca released in the first seconds is recaptured the factor regains control over the splitting of ATP and relaxation sets in, just as it does in KCl induced contractures.

(3) It follows from (1) and (2) that the power output even at extremely high rates of thawing will always be nugatory in comparison with, say that of a tetanus in living muscle or of the ATP-induced contraction of fiber models, because of the inevitable spontaneous relaxation of the thawing fibers. In this connection, it is only necessary to compare the maximal power in thaw contracture of ~ 400 g cm./min./g with the maximum in glycerinated psoas bundles which can reach the very high initial value of 10 000 g cm./min./g (Bendall, unpublished) When differential rates of thawing also play a part, as in thick strips, the power will be even more drastically reduced.

b The Rigor Phase The contracture aspect of thawing is the primary

event and is followed only later by loss of extensibility In the case of thick muscles (e.g Fig 10) this loss is rather ill-defined, beginning sometimes at about the half stage of the contracture and sometimes later and reaching completion only when the ATP level has decayed almost to zero This ill-defined onset is undoubtedly due to the differential rates of thawing throughout the muscle mass. In thin, rapidly thawing bundles, on the other hand, stiffening sets in more sharply at the end of the relaxation phase, showing that this is probably also the critical moment in the individual fiber bundles of the thicker muscles. In this respect, thaw rigor is quite different from the normal process, in which shortening, if it occurs, does so simultaneously with stiffening (Bendall 1951 Marsh, 1954)

Summarizing we can say that (1) The rate of thaw contracture and rigor are dependent largely on the rate of thawing (2) The chemical pattern is similar to normal rigor but unfolds much more rapidly, due to activation of the myofibrillar ATPase. (3) Contracture occurs together with the chemical changes immediately on thawing, and is complete before the ATP level has significantly decayed. In thin muscles, it is rapidly succeeded by relaxation and only later by loss of extensibility (4) It is to be concluded that thaw contracture is a special case of contracture in general and closely resembles the KCl induced contracture of phasic muscles. In contractures of this type, the stimulus is provided by upsetting the salt balance, the contracture ceasing as soon as this balance re-equilibrates. (5) There is strong evidence that it is primarily the inactivation and reactivation of the Marsh relaxing factor which brings about contracture and relaxation, respectively possibly through the primary release and subsequent recapture of bound Ca^{++} on thawing

B Delayed Rigor in Whale Muscle

At the opposite extreme to the very rapid rigor occurring during thawing is the much delayed rigor which frequently occurs in whale muscle. The detailed chemical and physical events in such a case were described by Marsh (1952b) and later by Webster (unpublished observation) A characteristic example is given in Fig 13, from Marsh (1952a) where the physical change was measured by means of a penetrometer

The most noticeable features are first, the very long delay before the

pH or ATP begins to fall rapidly, and secondly, the abrupt onset of the rapid phase, during which the rates of change are similar to those in rabbit muscle. At first sight, this might suggest that although the splitting of ATP was occurring from the moment of death, the level of ATP was being maintained exclusively from the P-C kinase reaction until the supply of P C failed, at which stage, glycolysis was stimulated and decay of ATP began in the normal manner Further investigation by Webster however, showed that this was not the case, but that on the contrary, the P-C level also remained high and static during the

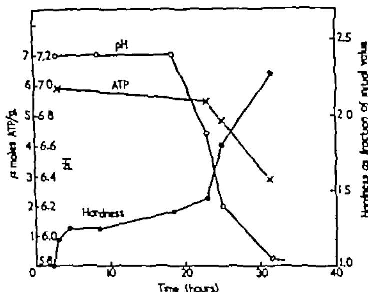


FIG. 13. The onset of rigor mortis in whale muscle, replotted from Marsh (1952a)

delay period. Similarly resynthesis of ATP by the oxidative pathway could be ruled out, (a) because the experiments were performed under anaerobic conditions and (b) because any oxygen absorbed initially would be used up long before the onset of the rapid phase (Marsh, 1952a) Thus, we can only conclude that ATPase activity was *more or less* completely inhibited for at least 20 hours after death, and then was suddenly stimulated by some unknown change within the muscle. One possible explanation is that the granular ATPase of the whale sarcoplasm shows the latency phenomenon, referred to above, (*Section IV C*) to a remarkable degree, and only becomes fully active

as the pH of the muscle slowly falls. In that case, we would merely have to postulate that the slow portion of the glycolysis curve is qualitatively the same as that in rabbit muscle but that the latency is initially more pronounced. Alternatively we might postulate that a fall in redox potential is sufficient to abolish the latency since we know that most ATPases and ATP kinases possess labile SH groups (Bailey and Perry 1947) which might be in the inactive S-S form at high redox potentials, and in the active SH form at low. Neither of these explanations is entirely satisfactory however, and it is evident that more detailed data are required before a convincing interpretation is possible.

In passing it may be noted that the only report of a similar delay in rigor in other species is that of the decapitated man, referred to in the introduction, where "irritability" and flaccidity of the musculature were demonstrated 20 hours after death (Taylor 1910)

C. Rapid Rigor in "Degenerated" Muscles

A naturally occurring phenomenon of some interest is the so-called total muscle degeneration of pigs (total M D) which seems to be caused by lowering of the ACTH level (Ludvigsen, 1956) This is characterized by a post rigor histological picture very similar to that obtained during thaw rigor (cf. Plate III) where the muscle fibers appear detached from their neighbors and there is much edema of the interstitial tissues. Particularly noticeable is the absence of cross striations. These results suggest that the main effect is on the sarcolemma which becomes abnormally sensitive to the pH fall during rigor and so allows a massive exodus of sarcoplasmic fluid. This is confirmed by the large increase in serum K from 22–24 mg % in the living animal to 52–87 mg % post mortem.

As might be expected in a case where the sarcolemma has deteriorated, with consequent activation of the myofibrillar ATPase the pH changes in the muscles after death are greatly accelerated and since pH is a good index of the ATP level, we may take this to indicate an equally precipitate onset of rigor For example, Ludvigsen (1956) gives average values for the pH of the M. gracilis, psoas, and l dorsi of normal pigs, taken 30 min. after slaughter of 6.5 6.1 and 6.4 respectively and of total M D pigs of 5 4 5.5 and 5.3 respectively There is little doubt that the normal muscles were not in rigor at the time of sampling whereas the degenerated muscles were

It would be of great interest to know just how much the degenerated

muscles shortened during rigor, in order to assess the extent to which the myofibrillar enzyme had been stimulated in such cases, although, even without such data, the high rates of fall of pH indicate that considerable activation of this enzyme must have occurred In this respect, the condition would closely resemble thaw rigor although we should have to suppose here that it was the pH fall during killing which brought about the primary destruction of the sarcolemma, followed by exodus of K release of bound Ca^{++} and consequent activation of the contractile proteins. Further study of the post mortem decay of P C and ATP in degenerated muscles would clearly be of great value.

V A THEORY OF THE RIGOR PROCESS

We have seen that theories of rigor developed before the "ATP-era" were incomplete, largely because they were based on the assumption that lactic acid was itself the primary cause, in spite of experimental evidence to the contrary It is now possible to construct a reasonably plausible hypothesis

Taking first the physical aspect, the evidence of studies of isolated fibers and their constituent proteins (Szent-Györgyi 1945 Bailey and Perry 1947 Weber and his school, 1952 1956 1957 Hanson and Huxley 1955 1957) overwhelmingly shows that the main change is from an extensible system of myosin and actin chains, able freely to slide past one another to a rigid system in which the actin and myosin combine. In detail, the most satisfactory explanation of the resulting fortyfold decrease of extensibility is that the actin chains are discontinuous in the region of the H zone, where they are joined together by an easily extensible protein, as yet unidentified whereas the myosin chains run right through the A band, including the H zone (Hanson and Huxley 1957) Pre rigor the actin chains may be partly pulled out of the A band, with extension of the H zone material, and apparent lengthening of the I band As rigor ensues, the actin and myosin chains combine, and the extensibility falls to that residing in the newly formed actomyosin of the A band and the residual extensibility of the free actin chains of the I band Thus, rigor will result in nearly the same proportional decrease in extensibility whether the length of the muscle at the time is greater or smaller than the rest length. This hypothesis therefore, solves the two greatest difficulties of accounting for the forty fold decrease in extensibility and for the independence of this decrease on the length of the muscle.

From the chemical aspect, we know that the high pre-rigor extensibility of the muscle depends solely upon the level of ATP which acts as a *plasticizer to keep the actin and myosin chains apart* (Weber 1956) From this, it follows that the time course of stiffening will be determined by the balance between the splitting and the resynthesis of ATP Of the enzymes which split ATP, the chief is the actomyosin ATPase because of its very high potential activity and of the associated contractile effects. As we have seen however this enzyme remains entirely dormant under the control of the Marsh relaxing factor during the course of normal rigor and is only activated if the muscle membrane is depolarized in some way as in the case of thaw rigor Thus the slow splitting during the normal process must be attributed entirely to the sarcoplasmic ATPase or ATPases.

Opposing the splitting process and maintaining the ATP at a high level is the resynthesizing machinery which under the prevailing anaerobic conditions consists of the glycolytic cycle and the P C-phosphokinase system. Under optimal conditions that is in a well fed animal dying quietly these two mechanisms provide an ATP potential equivalent to $\sim$ 162 μA. P_i/g of which $\sim$ 140 μA. are due to glycolysis, and $\sim$ 22 μA. to P C. The presence of P-C is, however all important, in spite of its smaller contribution to the total potential. This is due to two effects (1) The ATPase activity is minimal in the high ranges of pH from 7 0 to $\sim$ 6.5 where a high P C content is usually found, (2) In the absence of P-C the ATP level cannot be maintained by glycolysis alone, probably because myokinase can then immediately attack the ADP formed in the splitting process and in the early stages of glycolysis itself A molecule of AMP will thus appear for every two ADP molecules lost, and will in turn be deaminated to IMP

Combining these effects we find the time required to destroy all the P C and to produce enough lactic acid to take the pH from 7 0 to 6.5 at 17° C. is $\sim$ 320 min., whereas the time required for the completion of glycolysis from this point down to pH 5 6 is $\sim$ 330 min., calculating from the average rate of splitting and the available ATP actual and potential. The all important difference between the two pH ranges is, however that ATP can be maintained at a high level in the high range, but not in the low

From the above calculations, we can predict the effect of various treatments on the rate of decay of ATP Thus if we starve the animal before death to reduce the muscle glycogen, and then kill it in an

immobilized state, the potential glycolysis will be reduced to a level equivalent to a final pH of ~ 6.5 with the result that the ATP will begin to decay just before the end of glycolysis at ~ 220 min. after death. On the other hand if we kill a well-fed animal without prior immobilization, the struggle at death will result in a low pH in the excised muscle, a loss of P-C and a partial loss of ATP and the ATP level will decay from the moment of death. Yet the resynthesizing potential will be equivalent to ~ 88 μmoles of ATP/g in the second case and to only ~ 71 μmoles/g in the first.

At the opposite extreme to the above examples where more or less glycolysis is possible, is the case of exhausted animals, where the muscles contain no glycogen at death. We then have a state where the ATP alone or in the presence of a reduced supply of P-C is exposed fully to attack by the ATPase, and cannot, therefore, last for more than ~ 120 min., even under optimal conditions and generally for not more than ~ 30 min.

These, then, are the time relations of the decay of ATP which are so closely dependent on pH that this parameter alone is a useful guide to the stage to which the process has come (Marsh 1953). The stiffening process itself is, on the other hand, not so simply described, because the loss of extensibility is dependent not only on the ATP level but also upon the pH. Thus the level of ATP when stiffening sets in is ~ 2 μmoles/g if the final pH can fall below 6.3 but rises to ~ 4 μmoles when the pH fall is restricted by limitation of the glycolytic potential. This was at first taken to indicate a complex relation between the rate of ATP resynthesis and stiffening but this is no longer a necessary assumption, since it is now known that in the presence of Marsh factor the ATP level required for plasticizing the actomyosin of the myofibrils is lower the lower the pH (Briggs and Portzehl 1957). It is obvious, therefore, that the relation of the critical level of ATP to the final pH during rigor is merely a reflection of this fundamental property of the contractile proteins. In practice this has the effect of disproportionately delaying rigor in muscles with a high glycolytic potential, i.e. those which will reach a low final pH.

These fundamental relations between the ATP content and the stiffening of normal intact muscles are seriously disturbed during thaw *rigor* where the effect of freezing and thawing is to activate the actomyosin ATPase, that is to inhibit control by the Marsh relaxing factor and thus to bring about a rapid contracture which develops

many times the power of the very restricted contracture of normal rigor It seems likely that this thaw contracture is stimulated in the first instance by the massive salt flux on thawing which has the effect of releasing small amounts of bound calcium, thus inhibiting the Marsh factor As the salts re-equilibrate, however we may suppose that the necessary conditions for recapture of the calcium are established, so that control is regained and the muscle relaxes, providing sufficient ATP is still available for plasticization of the actomyosin. Thus, thaw rigor provides an excellent illustration of what happens when the actomyosin ATPase is activated even to a limited extent, and shows by contrast how minute such activation must be during normal rigor even under optimal conditions for shortening Thaw contracture of phasic muscles, in the respect that it is spontaneously reversible and also in that it begins at high ATP levels and may be complete before any net loss of ATP has occurred, is, therefore, of the same type as KCl-or caffeine-induced contractures (cf. Sandow 1955 Fleckenstein *et al.* 1954), and differs entirely from the shortening of normal rigor On the other hand, the subsequent stiffening sets in at about the ATP level characteristic of normal rigor

We may confidently conclude that the normal process of rigor consists in the change from free filaments of actin and myosin to filaments of actomyosin, which occurs as ATP is lost from the muscle by splitting by the sarcoplasmic ATPase, without significant activation of the contractile machinery The time of onset of rigor therefore, depends entirely upon the detailed relation between the resynthesizing and splitting processes. The pendulum has, therefore, swung away from the old theory that the greater the lactic acid the quicker the stiffening, to its opposite, that the slower the stiffening the more lactic acid will eventually be produced

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CHAPTER IX

Histochemistry of Skeletal Muscle and Changes in some Muscle Diseases

E. B Beckett and G H Bourne

The last thirty years or so has seen the accumulation of a vast mass of information concerning the biochemical properties of the various components of normal striated muscle fibers. On the one hand the muscle mitochondria (or sarcosomes) have been shown to possess the same types of enzymic activity as the mitochondria of other tissues and on the other hand the structural and enzymic properties of the components of the contractile elements of muscle fibers have been fully investigated with a view to elucidating the processes involved in muscular contraction

The biochemistry of human skeletal muscle in pathological conditions has attracted far less attention, possibly because of the difficulty of obtaining suitably fresh material with which to work and possibly also because of the difficulty involved in the interpretation of results when structural changes, for example a large increase in the collagenous tissue have occurred in the muscle sample as a result of pathological processes

An attempt was made to overcome this latter difficulty by Dreyfus *et al.* (1954) by relating results to non-collagen nitrogen, and they observed that in progressive muscular dystrophy there was a decrease in phosphorylase and aldolase activity in striated muscular tissues. This lead, however, does not seem to have been taken up by other groups of workers, and so the information available concerning the biochemical changes in human muscular or neuromuscular disease is meager indeed.

Compared with the literature available on the biochemistry of striated muscle, there is very little to be found dealing with histochemical aspects of this tissue. Indeed, the only detailed study of muscle carried out in recent years concerning anything other than motor endplates was that of Dempsey and associates in 1946, in which a range of histochemical properties of the tissue was investigated.

Because of the paucity of observations recorded in the literature, it would perhaps be as well to strike a note of caution at this point. The account of muscle histochemistry to be given in this chapter must not be regarded as an authoritative collection of facts, since in many cases work on a particular aspect of the normal histochemistry of this tissue has been done by two or three groups of investigators at the most. Furthermore, practically all of the work on pathological human muscle is that of the present authors and has not yet been confirmed by other workers. This chapter should therefore be taken only as a preliminary discussion of the few known facts, and as a possible background for the assessment of future investigations.

Again, because of the dearth of facts in the literature, we shall include in this survey results obtained from histochemical examination of normal animal striated muscle as well as those obtained from normal human muscle, but in describing pathological changes we shall confine ourselves almost entirely to human material.

I. RESPIRATORY ENZYMES

A. SUCCINIC DEHYDROGENASE

Histochemical techniques for the detection of succinic dehydrogenase activity depend upon the reduction of a tetrazolium salt by hydrogen removed from sodium succinate by the enzyme. As a result, the almost colorless tetrazolium salt is reduced either to monoformazan, which is of a pinkish hue and gives a diffuse coloration in regions of low enzyme

activity or to blue mauve diformazan crystals where there is greater enzymic activity This type of method was first introduced by Seligman and Rutenburg (1951) and has since been modified and subjected to critical analysis by a number of workers, including Shelton and Schneider (1952) Padykula (1952) Rosa and Velardo (1954) and Nachlas *et al* (1957)

1 *Normal Muscle*

Skeletal muscle possesses a moderate degree of succinic dehydrogenase activity which seems to vary little from species to species, if man is not included The typical picture obtained is of a generalized pink background color due to monoformazan, plus a variable number of blue diformazan granules. These granules seem to be situated in rows between the myofibrils (Padykula, 1952) and to be present in greater numbers towards the edges of muscle fibers.

Not only does the number of diformazan granules vary in different parts of the thickness of a muscle fiber but different muscle fibers may also contain greatly differing numbers of granules (Rutenburg *et al.*, 1953) sometimes so much so that in transverse section a checker board effect is obtained (Fig 33) This varying content of succinic dehydrogenase activity is probably an inherent property of muscle fibers, since it exists in the embryo well before birth (Beckett and Bourne, 1958a) and in animals it appears to be characteristic of particular muscles.

In man, the amount of succinic dehydrogenase activity varies considerably from muscle to muscle according to anatomical site For instance in our work the deltoid appeared to have much more activity than the trapezius. But there also seem to be random variations in intensity It may be that these latter variations are due to age and/or sex differences, although one cannot yet be certain of this, owing to the limited series of specimens which has been examined

2 *Pathological Muscle*

In 1951 Humoller and associates, using a biochemical method, observed that denervation of rat gastrocnemius led to a decrease in its succinic dehydrogenase content

From our own work on human muscle biopsies taken from various types of muscular or neuromuscular disorders (Beckett and Bourne 1958a) this loss of respiratory enzyme did not appear to be very

striking in cases where, from the clinical diagnosis (e.g in polyneuritis, motor neuron disease, and peripheral neuritis) one might expect the muscular innervation to be at fault. Only in one case of such a disease which was of peroneal muscular atrophy was there a considerable fall in succinic dehydrogenase activity and in this case the picture was complicated by the fact that the patient was a child of 5½ years. The low reaction intensity here might have been due in part to the child's age, since we have observed that in fetuses the succinic dehydrogenase activity in muscle is considerably lower than that in adults, and it is not yet known at what age adult levels of activity are attained.

The general impression which we have gained from our work, which covered specimens from a variety of muscular and neuromuscular disorders including muscular dystrophies, polyneuritis and polymyositis, motor neuron disease carcinomatous myopathies and others is that a decrease in succinic dehydrogenase activity tends to occur in the course of these diseases, but that it is a secondary change following gross structural disorganization of the muscle fibers and their surrounding connective tissue In other words, where there is slight atrophy or hypertrophy of the muscle fibers, there is no histochemically observable change in the concentration of succinic dehydrogenase in them However where there is a gross change in muscle fiber size, often accompanied by a great increase in the amount of interstitial dense connective tissue and/or adipose tissue as is observed in some muscular dystrophies, there is a marked decrease in the enzyme activity detectable by histochemical means

In only one case did there seem to be a primary disruption of the succinic dehydrogenase system. This was a sample of muscle from a case of polymyositis in which there was little histological evidence of muscle fiber destruction, but in the center of each fiber there was an area of little enzyme activity surrounded by a broad outer band of greater reaction intensity (Fig 32) This would suggest a disruption of the respiratory system in the center of each muscle fiber but a similar picture is not always obtained in cases of this disease, so that it offers no explanation of the pathological processes involved.

There appears to be no correlation between the observed intensity of reaction and the clinical diagnosis, but one must be cautious about the interpretation of this, because of the variability of the reaction in normal muscle and also because of the rather limited number of specimens which have so far been studied.

B Cytochrome Oxidase

The G-Nadi reaction, known to be due to cytochome oxidase activity was first used for histochemical purposes by Moog (1943) in her study of this enzyme in the chick embryo It depends upon the production of indophenol blue at the sites of activity when fresh tissue is incubated in a solution containing α naphthol and *p*-dimethyl phenylene diamine hydrochloride. The indophenol blue fades fairly rapidly so that the technique is not very satisfactory The only observations which have been made on skeletal muscle seem to be those of the present authors (Beckett and Bourne, 1958a) In the course of this work the depth of blue coloration obtained in the sections was compared with that of standard dilutions of aqueous toluidine blue.

The results indicated that in animal and human muscle a diffuse blue coloration is obtained in the muscle fibers and that it has no particular localization In rat muscle, different muscle fibers may show different reaction intensities, but this was observed very rarely in human muscle.

As with succinic dehydrogenase activity that for cytochrome oxidase varies according to the anatomical site of the muscle. There appears to be no correlation between the levels of activity of the two enzymes either in normal or in pathological human muscle, but whether or not this is due to technical difficulties it is impossible to say The number of specimens used in the course of our work was too small for us to be certain whether or not there is a decrease in the activity of cytochrome oxidase in pathological muscle

II. ESTERASES

The term "esterase" in histochemistry covers simple esterases, i.e. enzymes which split esters of short chain fatty acids, lipases, i e. enzymes which split esters of long chain fatty acids, and cholinesterases which split various choline esters. However it has become clear in recent years that there exists a whole spectrum of esterases with varying degrees of substrate specificity so that one cannot clearly define three separate enzyme groups. For the purposes of this present discussion it is proposed to consider the esterases under two headings, i.e (a) simple esterases and lipases and (b) cholinesterases, but merely because it is convenient to do so from the point of view of techniques which have been used for the study of muscle It is very necessary however to bear in mind the overlap in substrate specificity which exists between the groups.

A. Esterases and Lipases

The Gomori Tween technique for the demonstration of lipases was introduced in 1945 (Gomori, 1945) In this technique, a Tween substrate, i.e. a long-chained fatty acid ester of sorbitan or mannitan in which the remaining hydroxyl groups are etherified with ethylene oxide side chains of varying lengths, is split by the enzyme present in sections of acetone fixed tissue The free fatty acid so released is precipitated as its calcium salt at the site of enzyme action, then converted to its lead salt, and finally to brown lead sulfide, which is clearly visible under the microscope

A few years later (1949) a technique for the demonstration of simple esterases was elaborated by Nachlas and Seligman (1949a, b) In their method, the simple esterases of acetone fixed tissue split β-naphthol acetate, and the free naphthol which was released combined with a diazo dye to give a colored end product. The technique suffered somewhat from diffusion artifacts, and was later modified in several ways to improve localization. Gomori in 1950, and 1952 introduced α naphthyl acetate and naphthol AS acetate, respectively as substrates (Pearse, 1953a b) commercially available diazotates were used and for some tissues it was found that brief formalin fixation yielded better results than the originally used acetone fixation.

An alternative method for the demonstration of simple esterases was that of Barrnett and Seligman (1951) Their technique made use of sections cut from fresh or lightly formalin fixed tissues. The esterases in the tissues split indoxyl acetate, so releasing free indoxyl which was subsequently oxidized by the oxygen of the air to form insoluble crystals of indigo This method was criticized by Gomori (1952a) on the grounds that the aerobic oxidation of indoxyl to indigo is a slow process, and that because of this, the localization would be poor By 1954 however Holt and others (Holt, 1954) had modified the technique, not only by the introduction of 5-bromoindoxyl acetate or 5,5 diindoxyl acetate as substrate, but also by the use of copper ions and a ferro-ferricyanide oxidation reduction system to hasten the process of conversion of the enzymatically released indoxyl derivative to a compound of the indigo type. The localization of esterases demonstrated by this technique was immeasurably improved by these modifications in fact some of Holt's (1954) pictures of rat motor end plates rival the best which have so far been obtained with any available histochemical method.

All of these histochemical techniques for the demonstration of lipase and esterase have been used to some slight extent for the study of muscle, usually in conjunction with work on other tissues.

Gomori (1946) Richterich (1952) and Buño and Mariño (1952) all using the Tween technique on acetone-fixed material, noted that the skeletal muscle for several animals, including man, always gave a negative reaction. Nachlas and Seligman (1949b) obtained the same result in human muscle with their original β-naphthol acetate method. Chessick (1953) on the other hand, found a positive reaction for α naphthol esterase and naphthol AS esterase in the motor end plates, and possibly also in the muscle spindles, of mouse muscle after acetone or acetone-alcohol fixation The skeletal muscle of cat, rabbit, rat, and man, however gave a negative result with these substrates.

Denz (1953) using the β-naphthyl acetate method observed staining of rat motor end plates which was inhibitable with eserine and in addition, a diffuse noninhibitable esterase reaction in the muscle substance, whereas with the Gomori Tween technique he could only obtain the diffuse staining of muscle fibers Both of these procedures were carried out on fresh frozen sections.

Other workers have also described the presence of esterase activity in fresh or lightly formalin-fixed rat muscle. Barrnett (1952) using the original indoxyl acetate method obtained a positive reaction in both the striated and the smooth muscle of this animal and Pearson and Defendi (1957) observed high levels of esterase activity in rat motor end plates with either the 5-bromoindoxyl acetate method or the α naphthyl acetate or naphthol AS acetate techniques. These latter workers also found that reaction intensity obtained with a given technique depended upon pH The greatest activity with 5-bromoindoxyl acetate as substrate was observed between pH 4 8 and pH 5.8 whereas the highest degree of activity with α naphthyl acetate or naphthol AS acetate as substrates could be seen in a higher pH range i.e. pH 7.3 to pH 8 4

From these studies of the esterase activity of skeletal muscle it appears that the enzyme(s) is present, particularly in the motor end plates, but that it is inhibited in most animals by acetone fixation and/or paraffin embedding It also seems that the intensity of reaction obtained depends upon the pH used for a given technique

No work appears to have been done with histochemical techniques for lipase and esterase in relation to human muscular or neuromuscular disorder



PLATE I

B. Cholinesterases

The subject of cholinesterases and motor end-plates is dealt with in full by Couteaux elsewhere in this book, so we shall confine ourselves to a brief discussion of histochemical techniques for this enzyme and to a few remarks about cholinesterase positive structures in human muscle and their changes in some pathological conditions.

There are three types of technique available for the demonstration of cholinesterases.

The simple esterase techniques described above, and particularly the various naphthyl acetate methods, have been used from time to time but rarely for the study of muscle. Since these techniques primarily demonstrate simple esterases it is essential to use eserine in conjunction with them in order to differentiate between simple and cholinesterase and to use DFP (diisopropylfluorosphosphonate) to distinguish between the so-called "true" and "pseudo" cholinesterases. It must, however be remembered that these classifications according to inhibitor sensitivity are purely arbitrary and that the histochemical results for a given site or a given tissue may not correspond with the biochemical findings. A good review of this subject was published by Gomori and Chessick (1953)

A second type of technique which has been used for the demonstration of cholinesterases is Gomori's (1948) myristoyl choline method and its variations In this technique a long chain fatty acid is released from the substrate by the action of the cholinesterase and then combines

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Fig. 1 Acetyl cholinesterase at musculo-tendinous junctions in normal muscle The structures present here may represent stretch receptors. Magnification approx. × 150

Fig. 2. A "classic" motor end-plate from a case of motor neuron disease. Magnification approx. × 1,000

Fig. 3. Very atrophied muscle fibers in a sea of dense connective tissue from an advanced case of facio-scapulo-humeral dystrophy showing numerous *remaining* end-plate structures Magnification approx. × 150

Fig. 4 Single muscle fiber with its end-plate surrounded by dense connective tissue from the same case of facio-scapulo-humeral dystrophy Magnification approx. × 300

Fig. 5. A large end-plate on an isolated hypertrophic muscle fiber from a case of pseudohypertrophic dystrophy in which the muscular tissue had been almost entirely replaced by fat. Magnification approx. × 150

Fig. 6 End-plates, apparently normal in form, in muscle taken from a case of familial dystrophy Magnification approx. × 100.

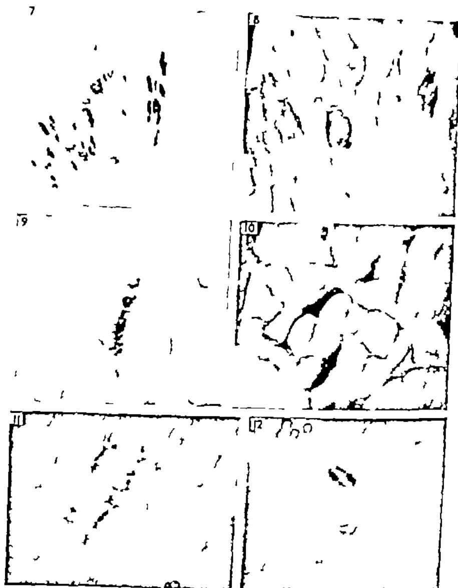


PLATE II

FIG. 7 A group of end-plate "gutters" in muscle from a case of facio-scapulo-humeral dystrophy to show well-marked transverse lamellae. Magnification: approx. × 1 000.

FIG. 8. Linear spiral structure with high concentrations of cholinesterase at intervals seen in muscle from a case of thyrotoxic myopathy. This might be a sensory nerve ending, e.g. stretch receptor. Magnification approx. × 150

with cobalt, and so is precipitated as a cobalt soap at sites of activity This cobalt soap is then converted to microscopically visible cobalt sulfide by the action of ammonium sulfide.

The introduction of this technique represented an advance on the use of the simple esterase methods, since it did at least utilize an ester of choline, but Gomori himself realized that it was far from specific and often failed to give a result at sites which were known to contain large amounts of cholinesterase for example the electric organ of the electric eel (Gomori, 1952b) This technique has been used by Hard and his co-workers (Hard, 1950 Hard and Hawkins, 1950 Hard and Peterson 1950) for work on the central nervous system of the dog It has hardly ever been used for muscle, since it tends to stain muscle nuclei, a possible artifact, and moreover it is difficult to interpret the structures demonstrated at motor end plates which may also be nuclei.

The most commonly used type of histochemical method for the demonstration of cholinesterase is that based on the hydrolysis of acetylthiocholine and butyrylthiocholine. The former compound was shown by biochemical means to be split by cholinesterases at a rate greater than that for acetylcholine, and was first employed for histochemical purposes by Koelle and Friedenwald (1949)

The original Koelle and Friedenwald technique was rather cumbersome and it has since been modified by several workers, including Koelle (1951) Gomori (1952c) Couteaux and Taxi (1952) Cöers (1953a) and Gerebtzoff (1953) The last three modifications involved the use of formalin fixation and acetate buffers at different pH levels to secure better morphological localization. More recently Bull *et al* (1957) used alcoholic ammonium sulfide instead of the usual aqueous solution to attain the same end.

FIG. 9 Acetyl-cholinesterase in an extended dotted" motor end-plate seen in muscle from an amputated leg This specimen showed signs of disuse atropy but the end-plate is probably normal.

FIG. 10. Muscle from a case of polymyositis showing the presence of acetylcholinesterase in the substance of the muscle fibers and the increase in concentration in atrophied fibers.

FIG. 11 Acetylcholinesterase in a dotted" spiral structure which may represent a normal end-plate seen in muscle taken from a case of thyrotoxic myopathy

FIG. 12. Acetylcholinesterase in a cake-frill or "palisade type structure. This was seen in muscle from an amputated leg, but is probably normal and may represent some sort of stretch receptor

The thiocholine techniques are undoubtedly the best for the demonstration of cholinesterases, both from the point of view of specificity and of precise localization. The localization obtained in motor end plates has been sufficiently good to allow studies of the fine morphology of the subneural apparatus of these structures to be carried out.

When the thiocholine technique is carried out, the acetyl or butyryl group is split off from the substrate by the enzyme and the thiocholine moiety combines with copper ions in the incubating medium, to be precipitated as copper thiocholine or copper thiocholine sulfate (Malmgren and Sylvén 1955) at sites of enzyme activity It is then converted to brown copper sulfide during the process of visualization.

1 *Cholinesterase in Normal Human Muscle*

Cöers (1953b, 1955) described two types of motor end plate to be found in human adult muscle firstly the "terminaisons en plaque, in which the "gutter" of the subneural apparatus is arranged like a continuous rope twisted into a complex pattern, and which have been observed in many different types of animal including the rat, mouse, lizard and goat and secondly the "terminaisons en grappe," which are composed of small islets of subneural apparatus grouped together but without any apparent connection between them. These latter vary considerably in size, since the number of islets of which they are composed may be three or four or may be thirty to forty and they seem to be characteristic of human muscle. Although in our recent work (Beckett and Bourne, 1957) we were using Gomori s modification of the thiocholine technique, which differs somewhat from the method used by Cöers, we were able to confirm his observations and also to confirm the presence of cholinesterase-positive motor end-plates at the poles of muscle spindles.

In addition we noticed that of the cholinesterase-positive structures present in muscle, only the terminaisons en plaque," or as we have called them the "classic motor end plates," ever gave a reaction when acetylthiocholine was replaced by the butyryl compound. This was true of both normal and pathological samples of muscle

As well as the structures just mentioned which are almost without doubt to be considered as motor end-plates, a number of other cholinesterase positive objects have been seen in both animal and human skeletal muscle.

In 1953 Coute[illegible] ed in the [illegible] g mouse and fish,

structures consisting of typical subneural apparatus "gutters" situated over the ends of muscle fibers at musculo-tendinous junctions. The "gutters" projected like parallel fingers into the muscle substance. Between then and 1956 this type of structure was also seen in a variety of other animals by Gerebtzoff and his co-workers (Gerebtzoff 1956) A little later we confirmed their existence in human muscle (Beckett and Bourne, 1957) and suggested that they might be stretch receptors.

In the course of our investigations a range of other structures was seen which we consider to be normal although they were not always observed in normal human muscle. Possibly earlier workers had missed them because their material had been formalin fixed It is known that formalin has a varying inhibitory effect on cholinesterase (Taxi 1952 Couteaux and Taxi, 1952 Beckett and Bourne, 1957) and from our preparations it appeared that these previously unreported entities contain less cholinesterase activity than the "terminaisons en plaque" or "terminaisons en grappe." With less sensitive variations of the technique, therefore, they may well have remained invisible.

In some of our muscle samples, objects were observed which consisted of a system of parallel "gutters" lying in the same direction as the long axis of the muscle fiber The "gutters" were arranged rather closer together at the center of the structure than at its edges, so that the whole looked rather like a cake-frill or wheat sheaf These structures could occur singly on a muscle fiber or there could be two on adjacent muscle fibers. Sometimes they were seen at points where muscle fibers ended in the middle of a fasciculus. One cannot be certain what their function could be, but their appearance would suggest that they might be stretch receptors.

In other muscle specimens, spirals of a continuous or discontinuous "gutter" were observed winding round single muscle fibers. These again may very well represent sensory nerve endings. Yet other entities were seen which were probably motor end-plates and not sensory endings composed of islets of "gutter" structure arranged in a band lying transversely across muscle fibers. This contrasts with the longitudinal arrangements observed by Coers. There were also some similar bodies of parallel "gutters" aligned at right angles to the long axis of the muscle fibers.

In addition to cholinesterase present in specific structures in muscle fibers, our observations (Beckett and Bourne 1957) have indicated that the enzyme is present in the muscle substance If unfixed frozen

sections are incubated in a medium containing acetylthiocholine iodide, and are visualized in the normal fashion, it is found that they have an overall deep yellow brown coloration, suggesting the presence of cholinesterase. This is confirmed by the fact that the depth of color is even, i e. it is not intensified at all in regions containing motor end-plates, and it does not vary in depth according the number of end plates present. Indeed the coloration is no less intense when there are no end plates at all in the section. If sections are incubated in a medium containing acetylthiocholine and $10^{-4}M$ eserine or in a medium containing butyrylthiocholine instead of the acetyl compound then there is virtually no coloration of the muscle fibers.

2 *Cholinesterase in Pathological Human Muscle*

The most thorough work which has been done on changes in the motor end-plate in human pathological muscle is undoubtedly that of Cöers (1955) in which he used the methylene blue and Bielschowsky silver techniques in addition to the thiocholine cholinesterase technique, in order to study the changes occurring in some muscular and neuromuscular disorders.

Cöers general conclusions from the study of forty-odd biopsies and some post-mortem material were as follows

In muscular atrophies, for example peroneal muscular atrophy of Charcot Marie-Tooth, no end plates were present in atrophied fibers, but in dystrophies (a heading under which Cöers includes myopathies, myotonic dystrophies, and myotonia congenita) there were good terminal arborizations as demonstrated by methylene blue. In children there was no change in the form of the subneural apparatus, but in adults there was an increase in the size of end plates.

In addition Cöers found that in most of the abnormal muscle studied, the size of the end plates in atrophied fibers was reduced whereas in the hypertrophied fibers seen in cases of dystrophy or partial neurogenic degeneration, there were large extended end plates. In contrast to this however Cöers observed that in certain muscular atrophies, and particularly in dystrophia myotonica the end plates were very extended and complex even where the muscle fibers were strongly atrophied. Further details of this work have been published recently (Cöers and Woolf 1959)

Our own work (Beckett and Bourne, 1957) has confirmed some of Cöers observations. The main object of our studies was to see whether

or not there was any decrease in the amount of cholinesterase present in the end plate areas of muscle taken from a variety of muscular disorders, including dystrophies of different sorts, motor neuron disease, carcinomatous myopathies, and neuropathies of unknown or obscure etiology For this reason, we used a thiocholine technique [Gomori's (1952c) modification] which did not employ formalin fixation prior to incubation with the substrate, so that the morphological picture suffered somewhat from diffusion. However it is possible to say something about the changes in morphology associated with muscle pathology

Firstly it must be said that the variety of normal structural forms of human subneural apparatus makes any pathological changes difficult in the extreme to detect with any certainty For this reason too it is a fallacy to carry out experiments, e.g denervation experiments, on animals and use these results to interpret the picture seen in pathological human muscle.

In the specimens which we examined there appeared to be no loss of cholinesterase activity at motor end plates, but in two cases, one of peroneal muscular atrophy and one of polyneuritis, there was a decrease in the amount of this enzyme present at the musculo-tendinous junctions. Furthermore, except in one case of peroneal muscular atrophy there did not seem to be any decrease in the number of cholinesterase positive structures present. In one or two cases, including one of pseudohypertrophic muscular dystrophy one of facio-scapulo-humeral muscular dystrophy and one of polymyositis, there may have been some break up of the structure of the subneural apparatus, but it is difficult to be sure of this. In one of the "control" specimens which was taken from the iliacus during cup arthroplasty of the hip, there seemed to be evidence of disruption of normal end plate structure since there were present numerous small dots with gutter structure about the size of the nuclei scattered over the muscle fibers.

One of the most striking things observed in our series of specimens was that where there was extreme muscle fiber atrophy accompanied by replacement of muscle fibers by dense collagenous tissue such as is seen in cases of facio-scapulo-humeral dystrophy or familial dystrophy the remaining fibers were smothered in pieces of end plate structure. This tremendous concentration of end-plate material was also seen in cases of pseudo-hypertrophic muscular dystrophy where there was disruption of muscle fiber structure and a high degree of infiltration by fatty tissue between the remaining muscle fibers. These observations

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Fr m what has already been said here, it can be seen that no clear
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of human muscle in the course of muscular or neuromuscular
a great deal more work must be done on normal human
n der to try and understand the miscellany of cholinesterase
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III. PHOSPHATASES

NON SPECIFIC ALKALINE PHOSPHATASE

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the aim of reducing diffusion artifacts. In these diazo dye techniques
(except for that of Loveless and Danielli) a naphthyl phosphate is split
by alkaline phosphatase and the released naphthol combines with a
diazo dye to give a colored compound.

Although these latter techniques offer some advantages, for example,
there is no confusion between end-product and preformed calcium,
they all suffer to some extent from diffusion artifacts, and there is evi-
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1 *Alkaline Phosphatase in Normal Muscle*

The biochemical work of Kay (1928) indicated that there was no
alkaline phosphatase in human muscle, and some of the later histo-

chemical work (Gomori, 1939 Dempsey *et al.*, 1946 Rossi *et al.* 1954) seemed to indicate that this was also true of muscle taken from a variety of animals, even when, as in the work of Dempsey Wislocki, and Singer prolonged incubation periods were used in order to try to obtain a positive result.

Other investigations using one or other of the techniques for alkaline phosphatase, however showed that although the fibers of skeletal muscle tissue were themselves negative, both the walls of capillaries and the endothelial lining of larger vessels were positive. This was found to be so in the muscle of various animals, including man by Gomori (1941b) in that of adult man and adult and embryonic mouse by Kabat and Furth (1941) and in that of adult man and rat by Newman *et al.* (1950b) Similar observations were made in human muscle by Manheimer and Seligman (1948) in human fetal muscle by Rossi *et al.* (1954) and also by McKay *et al.* (1955) and again in adult human muscle by Beckett and Bourne (1958b) These last workers also mentioned that the positive reaction does not extend throughout the whole blood vessle bed of a given area of muscle and suggested that physiological changes in the capillaries, e.g permeability changes, might find their reflection in the level of alkaline phosphatase activity in their walls.

Zorzoli and Stowell (1947) claimed that alkaline phosphatase was present in the nuclei, cross-striations, and the myofibrils of muscle as well as in the capillaries and surrounding connective tissue but their incubation periods were so excessively long (up to 70 hours), that grave doubt must exist about the validity of their results.

In recent years attempts have been made to localize phosphatases in fresh frozen sections in order to eliminate the effects of alcohol or acetone fixation However using such a technique Maengwyn Davies and Friedenwald (1950) could obtain no reaction in muscle using glycerophosphate as substrate even after 48 hours incubation. George Nair and Scaria (1958) claim that alkaline glycerophosphatase activity may be demonstrated in fresh frozen sections of pigeon breast muscle after prolonged incubation.

2. *Alkaline Phosphatase in Pathological Muscle*

In this section, we can only discuss the observations made in the course of our own work (Beckett and Bourne, 1958b) These can be regarded as preliminary indications only but may nevertheless be

suggest that the presence of the end plate or of some chemical substance such as the enzyme in it has a protective influence on the surrounding muscle tissue and prevents or delays its destruction. That this effect, however, is not due to the cholinesterase is suggested by the fact that in the muscle substance itself, the enzyme is increased in quantity *in muscle fibers showing evidence of atrophy and necrosis.*

From what has already been said here, it can be seen that no clear cut picture has emerged of the changes which occur in the subneural apparatus of human muscle in the course of muscular or neuromuscular disorders. A great deal more work must be done on normal human muscle in order to try and understand the miscellany of cholinesterase positive structures present in it before it is safe to argue about what constitutes a normal human end plate and to decide what changes are due to disease processes.

III. PHOSPHATASES

A. Non-Specific Alkaline Phosphatase

Two types of technique are available for the histochemical demonstration of non-specific alkaline phosphatase (a) the Takamatsu-Gomori method in which sodium glycerophosphate is split in the presence of calcium ions to yield calcium phosphate, and the calcium phosphate is then visualized either by the use of silver or by converting it to cobalt phosphate and thence to black cobalt sulfide and (b) the diazo dye techniques, first introduced by Menten and associates (1944) and later modified by Manheimer and Seligman (1948) Loveless and Danielli (1949) and Gomori (1951) among others with the aim of reducing diffusion artifacts. In these diazo dye techniques (except for that of Loveless and Danielli) a naphthyl phosphate is split by *alkaline phosphatase, and the released* naphthol combines with a diazo dye to give a colored compound.

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2 *Alkaline Phosphatase in Pathological Muscle*

In this section, we can only discuss the observations made in the course of our own work (Beckett and Bourne, 1958b) These can be regarded as preliminary indications only but may nevertheless be

worthwhile recording The results obtained unfortunately do not show a pattern of change associated with particular clinical diagnoses, so that no general conclusions can be drawn

In contrast to the picture seen of alkaline phosphatase in normal muscle, a sample taken during cup arthroplasty of the hip for osteo-arthritis, which showed signs of degeneration in some areas, contained small stellate cells with alkaline phosphatase in their cytoplasm and nucleoli. The nature of these cells is unknown, but they were not seen in any other specimens of diseased muscle.

Occasionally among the samples of pathological muscle (i.e. muscle taken from cases of muscular or neuromuscular disorders) where there had been an increase of interstitial connective tissue, e.g in cases of facio-scapulo-humeral dystrophy or familial dystrophy there were a few fine scattered alkaline phosphatase-positive connective tissue fibers. These may have been newly formed fibers, since these have been shown (Bourne, 1943 Fell and Danielli, 1943) to contain this enzyme.

In other specimens, alkaline phosphatase was present in some of the muscle fibers. The occurrence of these enzyme-positive muscle fibers was not correlated in any way with the general histological state of the muscle or with the diagnosis. The samples showing alkaline phosphatase positive muscle fibers consisted of two out of five cases of facio-scapulo-humeral dystrophy one out of two cases of familial dystrophy one case of dystrophy with periodic paralysis, one case of pseudo-hypertrophic muscular dystrophy one case of possible pseudohypertrophic dystrophy possible polymyositis, one case of possible thyrotoxic myopathy and one out of three cases of motor neuron disease.

The enzyme positive muscle fibers were scattered and were usually in an atrophied state (although this was not so in the case of dystrophy with periodic paralysis) but did not seem to differ greatly morphologically from other atrophied fibers present. In nearly every case, the enzyme positive muscle fibers were surrounded and invaded by a network of fine reticular like fibers which also contained alkaline phosphatase, none of the surrounding connective tissue, however showed any evidence of a positive reaction.

B Acid Phosphatase

Two varieties of technique are available for the histochemical demonstration of acid phosphatase.

The glycerophosphate method, introduced by Gomori (1941a) involves the precipitation of the inorganic phosphate, released from the substrate by enzyme action in the form of lead phosphate, and its subsequent conversion to brown lead sulfide. This method is renowned for its waywardness, but, although the efforts of many workers (for instance, Newman *et al.*, 1950a Gomori, 1950 Goetsch and Reynolds, 1951) have been directed towards improving this state of affairs, no modification has emerged to make the technique reliable

The diazo dye methods are very similar to those for alkaline phosphatase, i e., they involve the enzymatic splitting of a naphthyl phosphate, but at an acid pH followed by the combination of the free napthol radical so formed with a diazo dye to give a colored reaction product. The first of these techniques was introduced by Seligman and Manheimer (1949) It has since been modified by Friedman and Seligman in 1950 Grogg and Pearse in 1952 and Burton in 1954 (see Rutenburg and Seligman, 1955) in order to make it technically more simple and to decrease the diffusion artifacts of the original method.

All of these diazo dye methods were simultaneous coupling techniques, i e., both the substrate and the dye were present in the mixture in which the sections were incubated. The recently introduced Rutenburg and Seligman (1955) technique represents a new departure from established practice for two reasons firstly these authors used sodium 6-benzoyl-2-naphthyl phosphate instead of the more usual naphthyl phosphates, and secondly that their technique is a post incubation coupling method, i e., the sections are first incubated with the substrate and then treated with a diazo dye solution afterwards. Using this new technique Rutenburg and Seligman claim that diffusion artifacts are virtually eliminated but, unfortunately the colored end product fades after two weeks or more.

Rutenburg and Seligman's view concerning the lack of diffusion artifacts produced by this technique is supported by some recent work by Defendi (1957) in which he observed that 6-benzoyl 2 naphthol has a strong affinity for various tissue components. It would therefore remain at the sites at which it was released by enzyme activity until coupled with the dye in the second stage of the procedure.

1 *Acid Phosphatase in Normal Muscle*

For the study of acid phosphatase in muscle, the Gomori technique seems to have been the one chosen by the various investigators.

Gomori s work (1941a) led to the conclusion that there was no acid phosphatase in human skeletal muscle taken either at biopsy or with in 4 hours post mortem, and this result was supported by observations on rat and monkey muscle by Dempsey *et al* (1946) Wolf and associates (1943) however obtained a positive reaction in the skeletal muscle of various adult animals. They found that the sarcolemma and muscle nuclei showed a moderately intense reaction and that the cross-striations were as a rule clearly visible. Sometimes, however the muscle fibers were unreactive.

In addition, Wolf *et al.* demonstrated that the axons, the nuclei, and the cytoplasm of Schwann cells, and the endoneurial cells of peripheral nerves were acid phosphatase positive that in arteries and veins all nuclei and smooth muscle cells were positive, and that in capillaries only the nuclei gave a reaction

A few years later Rossi *et al* (1953 1954) obtained a positive acid phosphatase reaction in the nuclei of human fetal muscle, and in their later paper stated that there was enzyme activity in the sarcolemmal nuclei of adult human muscle.

Our own observations (Beckett and Bourne, 1958b) support many of

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FIG. 13 Alkaline phosphatase in muscle from a case of facio-scapulo-humeral dystrophy Several of the few remaining muscle fibers give a positive reaction. Magnification approx. × 150

FIG. 14 Atrophied, alkaline phosphatase-positive muscle fibers, seen in a case of thyrotoxic myopathy Note the fine, enzyme-positive connective tissue fibers surrounding the muscle fibers. Magnification approx. × 150.

FIG. 15 A very much disrupted muscle fiber with alkaline phosphatase in the remnants. This was observed in a case of doubtful diagnosis (pseudohypertrophic muscular dystrophy or polymyositis) in a young boy Magnification approx. × 150.

FIG. 16. A non-atrophied alkaline phosphatase positive muscle fiber from a case of muscular dystrophy with periodic paralysis. Magnification: approx. × 350.

FIG. 17 Muscle from a case of familial muscular dystrophy showing a mass of acid phosphatase positive cells invading a muscle fiber Magnification approx. × 200.

FIG. 18. Muscle, again from a case of familial muscular dystrophy showing invasion of muscle fibers by acid phosphatase-positive connective tissue fibers. The very atrophied fibers present in this field contain much acid phosphatase. Magnification approx. × 200.

FIG 19 Acid phosphatase in muscle from a case of pseudohypertrophic muscular dystrophy The muscle fiber on the extreme left contains a high concentration of the enzyme and is being invaded by masses of cells. The larger fiber in the center contains pale-staining nuclei and the fiber to the right of this contains deeply staining nuclei. Magnification pprox. × 400.

FIG 20. 5-nucleotidase in the walls of blood vessels of muscle from a case of facio-scapulo-humeral dystrophy This is a normal distribution pattern for the enzyme.



PLATE III

the findings of Wolf *et al.* (1943). In specimens of normal muscle, the most reactive elements were the axons and the so-called neurokeratin of the peripheral nerves, the cell walls of adipose tissue, the fibroblasts of tendon, and also the groups of granules which are situated at the poles of the nuclei of both muscle fibers and connective tissue. These granules probably represent the Golgi apparatus. The nuclei of connective tissue, and of blood vessel walls, and some also of those in muscle fibers contained a moderate amount of the enzyme. The muscle fibers themselves, on the other hand, and the smooth muscle of blood vessel walls were less reactive. The cross striations of voluntary muscle were often visible. As Wolf *et al.* we sometimes found that the muscle cells were negative, but this was usually a reflection of the length of incubation used. Capillary walls were occasionally positive, the nuclei being more so than the cytoplasm of the endothelial walls.

Rutenburg and Seligman's (1955) work on formalin-fixed tissue indicated that their method produced a granular reaction for acid phosphatase in skeletal muscle. This reaction had no particular localization. It is difficult to reconcile this observation with the results obtained with the Gomori technique.

2. *Acid Phosphatase in Pathological Muscle*

Again for this section we must rely upon our own results and must emphasize that these have not yet been confirmed.

FIG. 21. Muscle from a case of motor neuron disease, showing a 5-nucleotidase positive capillary which has apparently "eaten" its way into the muscle fiber. Magnification approx. × 350.

FIG. 22. A very atrophied muscle fiber seen in a case of query pseudohypertrophic muscular dystrophy, query polymyositis. The fiber substance contains a high concentration of 5-nucleotidase and so also do the invading cells. Magnification approx. × 450.

FIG. 23. Muscle from a case of familial dystrophy of late onset, showing high 5-nucleotidase activity in atrophied muscle fibers and in the cells and fibers which are destroying them. Magnification approx. × 175.

FIG. 24. 5-nucleotidase in muscle from a case of facio-scapulo-humeral dystrophy. Note the enzyme activity in very atrophied muscle fibers, in capillaries, in the fibroblasts of the dense connective tissue, and in some of the connective tissue fibers. Magnification approx. × 100.

FIG. 25. 5-nucleotidase. Muscle from a case of motor neuron disease to show enzyme-positive capillaries invading a muscle fiber.

FIG. 26. 5-nucleotidase. A sample of muscle taken from a case of familial dystrophy to show a muscle fiber undergoing atrophy and at the same time acquiring 5-nucleotidase activity.



PLATE IX

In general there appeared to be little change from normal in the acid phosphatase activity of muscle specimens taken from cases of muscular and neuromuscular disorders. There was a reduced reaction, as judged from the incubation time needed to produce minimal coloration in three cases of periodic paralysis and one of peroneal muscular atrophy. There was a slight reduction in activity in one case each of polymyositis, motor neuron disease, and myopathy of unknown origin. Although we have listed these cases here, it must be said that the change in acid phosphatase activity of a given specimen is not linked with its clinical diagnosis.

A generalized increase in acid phosphatase activity was never observed but it was very noticeable that all atrophied fibers, whether or not they also showed signs of degeneration, contained more acid phosphatase activity than normal. This was very strikingly shown in peroneal muscular atrophy and motor neuron disease, where the atrophied fibers were situated in groups among the more normal muscle fibers.

Groups of inflammatory cells invading degenerating fibers often showed a moderate to strong histochemical reaction for acid phosphatase, although this type of distribution was not as striking as that seen in preparations demonstrating 5-nucleotidase activity. The invading cells were particularly obvious in cases of muscular dystrophy of different types and in cases of polymyositis. Occasionally, too, invading acid phosphatase positive capillaries were observed.

IV. 5-NUCLEOTIDASE

5-nucleotidase was originally studied by biochemical means. In 1934 (Gulland and Jackson, 1938) Reis found 5-nucleotidase in the retinas of various animals, and later work by this author (Reis, 1951) and also by Gulland and Jackson (1938) demonstrated that it was present in a variety of tissues taken from animals and also from the human body.

The histochemical techniques for 5-nucleotidase are of two types. The one evolved by Gomori, which uses acetone fixed material, arose from the study of phosphatase specificity (Gomori, 1949; Newman *et al.* 1950b). A second type of technique, with which comparatively little work has been carried out, utilizes fresh frozen sections of mammalian tissues (Maengwyn Davies *et al.*, 1952; Padykula and Herman 1955a, b). Both types of method are based essentially on the Gomori alkaline phosphatase technique but with the replacement of glycerophosphate by muscle adenylic acid.

A. 5-Nucleotidase in Normal Muscle

The apparent distribution of 5-nucleotidase in muscular tissue depends upon the technique used.

Newman and his co-workers (1950b) were unable to demonstrate 5-nucleotidase in acetone fixed muscle taken from a range of animals. Later work by the present authors (Beckett and Bourne, 1958c) showed that in normal adult human muscle there may be no 5-nucleotidase activity at all, or there may be a slight reaction for the enzyme. The reaction when present, is restricted to the nuclei and the intimas of vessels of larger size than capillaries, to the axons and so-called neurokeratin network of peripheral nerves present, to the connective tissue sheaths of muscle spindles, and to occasional fibroblasts Incubation periods of up to 24 hours, are however needed to demonstrate 5-nucleotidase in human muscle. This enzyme activity demonstrated at pH 8.25 is due to a specific 5-nucleotidase and not to alkaline phosphatase since if the substrate is replaced by sodium glycerophosphate a reaction is never obtained.

The distribution of 5-nucleotidase is curious since the enzyme is associated with only certain of the larger vessels, and this phenomenon does not appear to be due to technical difficulties. The physiological interpretation of this specific distribution is obscure.

In rat rectus abdominis muscle incubated for 6 and 8 hours at pH 8 0 this variable distribution in vessel walls is most marked. Those the size of arterioles and venules are especially affected, for quite commonly one of an adjacent pair shows an intensely positive reaction while its neighbor remains negative Capillaries are also positive to a variable degree and where they are strongly reactive diffusion to nearby muscle nuclei occurs and gives the latter a spurious positivity As with human material the muscle fibers are consistently negative. From the point of view of the amount and distribution of 5-nucleotidase, acetone fixed mouse muscle is more akin to human then to rat muscle.

It does not seem to be particularly easy to demonstrate 5-nucleotidase in fresh frozen sections of mammalian skeletal muscle Maengwyn Davies *et al* (1952) could obtain only a diffuse staining of rat skeletal muscle fibers after 72 hours incubation at pH 8.25 Some muscle nuclei and the adventitia and media of blood vessels also became positive under these conditions. Three years later Padykula and Herman (1955b) observed no reaction in lingual and leg muscle

after incubation for 30 minutes with muscle adenylic acid at pH 9.4. This latter negative result may in part be due to the high pH used, since we have observed that 5-nucleotidase activity varies considerably with pH (Beckett and Bourne, 1958c).

B. 5-Nucleotidase in Pathological Muscle

Usually in pathological muscle, the pattern of distribution of 5-nucleotidase activity was essentially the same as in normal samples, i.e., the reaction was absent or was limited to groups of blood vessels and to small areas of connective tissue in their immediate vicinity (Beckett and Bourne, 1958c).

In cases where there had been a considerable reduction in the size of muscle fibers present, with a concomitant increase in the amount of dense collagenous connective tissue (as is for instance, seen in facio-scapulo-humeral dystrophy or familial dystrophy) and especially where this was associated with inflammatory changes in and adjacent to the muscle fibers, there was frequently a very great increase in the amount of enzyme present. Where there was less widespread muscle fiber atrophy and necrosis, a smaller increase in the amount of 5-nucleotidase was observed.

Where the activity of this enzyme was increased, it was present in its usual sites, i.e. in the blood vessels, nerves etc., but the areas of connective tissue showing a positive reaction were considerably enlarged. When there was a lymphocytic infiltration of the connective tissue, the lymphocytes also contained 5-nucleotidase activity. In addition to this increased connective tissue reaction, some muscle fibers were enveloped and appeared to be actively eroded by inflammatory cells, reticular like fibers, and capillaries, all of which were enzymatically active. At the same time, the muscle fibers themselves accumulated 5-nucleotidase. The occurrence of this increase in 5-nucleotidase activity was not limited to neuromuscular and muscular disorders, since an essentially similar picture was seen in muscle taken from around the hip joint of a case of osteoarthritis of 5 years standing.

V. OTHER PHOSPHATASES

The literature concerning other phosphatases is sparse, particularly in connection with skeletal muscle tissue, and the results obtained in muscle with other phosphate substrates seem to depend primarily upon the technique used.

Zorzoli and Stowell (1947) found that at an alkaline pH fructose 1,6-diphosphate gave the same reaction distribution as sodium glycerophosphate in acetone-fixed paraffin-embedded skeletal muscle.

Three years later Newman *et al* (1950b) also using acetone-fixed muscle from several different animals, and carrying out their incubation at pH 9.2, observed that a wide variety of substrates, including glycerophosphate, glucose 1 phosphate, hexose diphosphate, creatine phosphate, yeast adenylic acid, yeast nucleic acid thiamine pyrophosphate, and barium phytate gave a typical alkaline phosphatase reaction in muscle, i.e. the endothelium of blood vessels was positive. All of these substrates and adenosine triphosphate and 5-nucleotide gave nuclear staining also but only after prolonged incubation periods

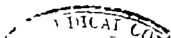
These results indicated therefore, that if specific phosphatases existed in fresh muscular tissue only a little adenosine triphosphatase and 5-nucleotidase activity could survive acetone fixation or at any rate, the remainder of the phosphatases were not demonstrable under the experimental conditions employed The only evidence to the contrary was that of Glick (1946) who using acetone-fixed paraffin embedded cockroach muscle, obtained a positive reaction with ATP but not with glycerophosphate.

Later work on the phosphatases of muscle was directed towards the development of methods utilizing fresh frozen sections in order to demonstrate specific enzymes, and particularly to demonstrate ATPase.

The early efforts of Maengwyn Davies and Friedenwald (1950) do not seem to have met with much success, since after 48 hours incubation they obtained a reaction limited to nuclei and blood vessels with glucose-1 phosphate but no reaction at all with hexose diphosphates, β-naphthyl phosphate, or sodium glycerophosphate.

Two years later the technique of these workers had been improved by the use of varying pHs and the introduction of inhibitors and activators to demonstrate the specificity of the phosphatases (Maengwyn Davies *et al* 1952)

Using the modified technique at pH 9 9 with ATP as substrate they found that after 24 hours incubation the muscle fibers were blackened and had darker staining nuclei These nuclei had a blackened membrane and then an unstained region between it and the chromatin. The sarcolemma and the endothelium of blood vessels also showed activity



At pH 8.25 a pH at which these workers supposed myosin ATPase to be active, the muscle fibers themselves and their sarcolemmas, and also the chromatin of muscle nuclei were intensely stained, after 22 hours incubation with ATP The endothelium of blood vessels of all sizes and the smooth muscle of the media were diffusely darkened. In addition the nuclei of the blood vessels were moderately positive, and activity was also observed in the various connective tissue sheaths of peripheral nerves. With creatine phosphate and yeast adenylic acid these workers could only obtain patchy reactions after very long periods of incubation (48–96 hours)

Further investigations on the technical side by Padykula and Herman (1955a) indicated that the sodium acetate used in the method of Maengwyn-Davies and her colleagues had a strong inhibitory effect on phosphatases, and by using a modified Gomori substrate at pH 9 4 Padykula and Herman obtained a positive reaction with ATP in 15–30 min. a much more satisfactory result than those of the earlier workers.

This positive reaction observed by Padykula and Herman was probably situated in the myofibrils, and there was no sarcoplasmic staining Blackening of cross-striations was sometimes seen and smooth

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FIG. 27 Sulfhydryl groups in an artery wall. The central area of each smooth muscle fiber which probably represents the nucleus, is lighter than the periphery Magnification × 350.

FIG. 28. Transverse section through muscle fibers from a case of facio-scapulo-humeral dystrophy showing that the concentration of sulfhydryl groups is the same in atrophied and hypertrophied fibers. An identical picture is seen in amino group preparations. Magnification × 170.

FIG. 29 Muscle taken from a case of periodic paralysis showing "shading" of reaction for sulfhydryl groups. Again, amino group preparations were identical in appearance. Magnification × 170

FIG. 30. Very atrophied, but otherwise apparently normal, muscle fibers from a case of peroneal muscular atrophy The concentration of sulfhydryl groups is unchanged, and this was true also of amino groups. Magnification × 170.

FIG. 31 Succinic dehydrogenase in gastrocnemius from a case of pseudohypertrophic muscular dystrophy Note the curious "woven" pattern of the diformazan granules, which reflects the pattern of alignment of the myofibrils. Magnification: approx. × 180.

FIG. 32. Succinic dehydrogenase in gastrocnemius from a case of polymyositis. Note the larger fibers with little reaction in them and the smaller fibers with a broad peripheral band of reaction. The central light zone possibly represents a primary biochemical lesion. Magnification approx. × 120

FIG. 33. Muscle from a rat, showing the checker-board effect of fibers with high and low concentrations of succinic dehydrogenase activity



PLATE V

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Fig. 33 Muscle from a rat, showing the "checker-board" effect of fibers with high and low concentrations of *succinic dehydrogenase activity*

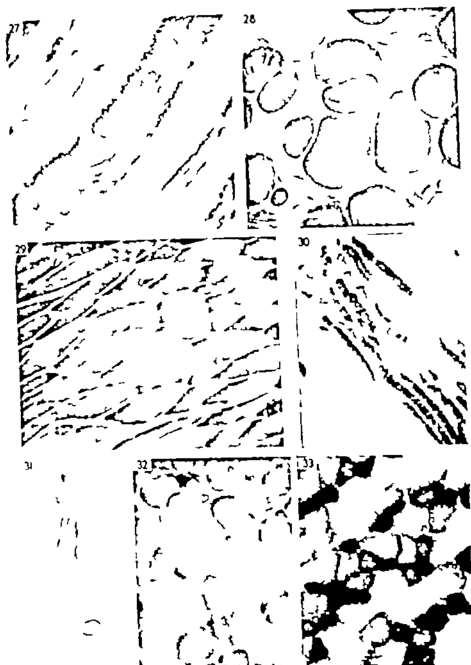


PLATE V

muscle and endothelium of blood vessels were also positive. In contrast to this, adenosine diphosphate or muscle adenylic acid gave little reaction under the experimental conditions used.

VI. GROUPS IN MUSCLE PROTEINS

A. Sulfhydryl and Disulfide Groups

Various methods have been devised for the histochemical detection of sulfhydryl and disulfide groups, and these have been the subject of a recent review by Gomori (1956) The one which has probably been most used for the study of striated muscle is the dihydroxy dinaphthyl disulfide (DDD) technique first introduced by Barrnett and Seligman (1952) for the demonstration of sulfhydryl groups, and later modified by the inclusion of thioglycollate reduction (Barrnett and Seligman, 1954) in order to detect disulfide groups as well. In this technique the substrate combines with sulfhydryl groups and at the same time splits into two parts. One part represents a by product of the reaction, and is removed by washing The second which is attached to the protein and which is a naphthol compound, combines with a dye such as tetra azotized diorthoanisidine, to give a colored end product. This end product is pinkish mauve where there are few sulfhydryl groups and monocoupling has occurred, but bluish mauve where there are more sulfhydryl groups and dicoupling has occurred.

1 *Normal Muscle*

According to Barrnett (1953) *in skeletal muscle there is an intense* reaction for sulfhydryl groups with no apparent differentiation between A and I bands, and no nuclear reaction. Similar results were obtained by us a few years later (Beckett and Bourne, 1958d) In human, rat and goat adult skeletal muscle the reaction intensity for sulfhydryl and disulfide groups was quite strong and there were no apparent species differences. The picture obtained seemed to indicate that all of the sulfur linkages in muscle were in the sulfhydryl form, since the slides showing sulfhydryl gro vere of the sa nsity as those showing sulfhydryl plus ups Th was limited to myofibrils and the sarco virtually cross-striations were visible, especially i em spec as of deeper reaction intensity were so near th ends of muscle fibers. heir significa ear

Like Barrnett (1953) the present authors observed a strong reaction for sulfhydryl groups in the smooth muscle of blood vessels and also found that the nuclei of these muscle fibers were less positive than the cytoplasm.

In peripheral nerves, Barrnett (1953) noted a reticulate network which was positive for sulfhydryl groups, and this was later confirmed by us (Beckett and Bourne, 1958d) Barrnett and Seligman (1954) however maintain that this reticulate network showed a reaction for disulfide groups only The reason for these apparent differences is not obvious. In the connective tissue between the muscle fibers, there is a slight positive reaction (Beckett and Bourne 1958d) the cellular elements being rather more deeply stained than the connective tissue fibers.

2. *Sulfhydryl Groups in Pathological Muscle*

The only work on the subject of sulfhydryl groups in pathological muscle appears to be that of the present authors. As judged by histochemical methods, we could find no change at all in the concentration or distribution of sulfhydryl groups in the range of muscular and neuromuscular disorders which were examined (Beckett and Bourne, 1958d) This was true even where there was gross change in the size of muscle fibers with or without necrotic changes, and where fibers were being replaced by adipose or dense collagenous tissue.

B Protein Bound Primary Amino Groups

The histochemical technique for the demonstration of these groups was first described by Weiss and associates (1954) and is based upon the use of 3 hydroxy 2 naphthaldehyde as substrate. The aldehyde grouping on this compound reacts with primary amino groups of the protein, and the naphthyl part of the molecule then reacts with tetra azotized diorthoanisidine to give a color very like that obtained with the technique for sulfhydryl and disulfide groups. As with this latter technique the color of the end product differs according to whether monocoupling or dicoupling has occurred.

1 *Normal Muscle*

The work of Weiss *et al* (1954) and of the present authors (Beckett and Bourne 1958d) indicates that the distribution of protein-bound

primary amino groups is very similar to that of sulfhydryl groups. In skeletal muscle fibers themselves the myofibrils have a moderate to strong reaction with fine banding due to cross-striations, and there is sometimes a darker reaction at the sides and ends of muscle fibres. The nuclei and sarcoplasm are negative or nearly so. The reaction in the smooth muscle of blood vessel walls is of about the same intensity as that in the striated muscle fibres. The interstitial connective tissue shows little reaction and the cells in it are less positive than in sulfhydryl group preparations. Similarly the reaction in nerve fibers is much less conspicuous than in the latter preparations.

2. *Amino Groups in Pathological Muscle*

The picture in muscular and neuromuscular disorders can be summed up quite simply by saying that, as far as our own observations go, there is never any change in concentration or distribution of primary protein bound amino groups. It must be emphasized however that these results are as yet unconfirmed by other workers.

VII. OXIDATIVE SCHIFF PROCEDURES

If tissue sections are treated with oxidizing agents, such as chromic acid, acidified potassium permanganate, periodic acid, or lead tetra-acetate, aldehyde groupings are formed which can then react with Schiff reagent to form a magenta color.

Chromic acid and acidified potassium permanganate were the first known oxidizing agents (Lillie, 1951) but the intensive use of oxidative Schiff procedures for histochemical work dates from the introduction for this purpose of periodic acid (Hotchkiss, 1948; McManus, 1946). The use of periodic acid, and the later introduction of lead tetra-acetate (Shimizu and Kumamoto, 1952) for the oxidation of tissue sections, has prompted intensive investigation of the chemical reactions involved in oxidative-Schiff methods. As a result, it has become apparent that as well as the 1,2-glycol groups previously known to be oxidized by the reagents mentioned above, other groups, chiefly substituted 1,2 glycol groups are also attacked (e.g. Glegg *et al.*, 1952a; Hale, 1957). Unsubstituted and substituted 1,2-glycol groups are present in a variety of tissue components including glycogen, mucopolysaccharides, mucoproteins, and some lipids, and all of these substances may be demonstrated by oxidative-Schiff techniques. Because of this, these

techniques have been modified to make them more selective for instance saliva or diastase (Bensley 1939 Lillie and Greco 1947 among others) have been used to remove glycogen, and lipid solvents (Leblond 1950) have been used to remove fats. Other workers (Lillie, 1951 Glegg *et al.* 1952a, b Casselman, 1954) have directed their attentions towards the possibilities of increasing the selectivity of oxidative-Schiff methods by careful choice of oxidizing agent and the conditions under which it is used

1 *As Applied to Paraffin Sections of Muscle*

This type of method has been applied to skeletal muscle by Dempsey *et al* (1946) who used a chromic acid-Schiff technique on sections of skeletal muscle taken from goat, man, and rat, and also by the present authors (Beckett and Bourne, 1958c) who used periodic acid or lead tetra acetate oxidation followed by Schiff's reagent on sections of normal and pathological human muscle.

The conclusions reached from the two sets of results were similar In both cases it was observed that stainable material could be present dispersed within the muscle fibers either as fine granules or in large irregularly distributed aggregates. In human muscle (Beckett and Bourne, 1958c) it appears that if the specimen is taken at biopsy or within an hour or so of death the aggregations of stainable material tend to be small, whereas at 12 hours post mortem the size of the aggregates has increased and by 48 hours post mortem they have disappeared.

In addition to this randomly distributed material, there is also staining of the cross-striations, which Dempsey *et al* (1946) claim is at the level of the I discs.

The work of the present authors indicates that the amount of stainable material in normal muscle is very variable and that there is no correlation between the amount present and the anatomical site of the muscle concerned In cases of muscular and neuromuscular disorder the amount of stainable material is also variable but the variations are within normal limits and there seems to be no correlation between the amount of stainable material present and the clinical diagnosis involved Even muscle fibers which show gross atrophy or hypertrophy appear to contain normal concentration of oxidative Schiff positive material

Both Dempsey and his colleagues and we ourselves have observed that

diastase (or saliva) will not remove all of this oxidative-Schiff positive material. Dempsey *et al.* found that the material in irregularly arranged aggregates was removable whereas that in the cross-striations was not. Their interpretation of this was that not all of the positive material was glycogen. In our experience, the picture was not as simple as this. Sometimes diastase would remove both types of stainable material, sometimes only that in the irregular aggregates, and sometimes none at all even if the sections were all incubated in the same diastase bath together. We would agree with Dempsey *et al.* (1946) that the material in aggregates is more readily attacked by diastase or saliva than that in cross-striations, but in view of our results we are inclined to think that all of the stainable material is glycogen, but that it is protected to varying extents in different sites by protein associated with it. However it would be unwise to be too dogmatic on this point, particularly as it is known that diastase preparations (which we have used instead of saliva for most of our work) are contaminated with variable amounts of proteolytic enzyme.

2. *As Applied to Gelatin Sections of Muscle*

In 1946 McManus first introduced his periodic acid-Schiff technique, and two years later he suggested (McManus, 1948) that it might be applied to formalin fixed frozen sections in conjunction with the Baker (1944) Sudan Black technique for fats. McManus was of the opinion that glycogen would not be stained in such sections. As

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FIG. 34. Muscle from a case of periodic paralysis to show distribution of fat in large streaks in some fibers. Magnification × 170.

FIG. 35. Muscle from a case of myopathy to show the great variation possible in the number of fat droplets in different muscle fibers. The dark dots at the periphery of the fiber indicate the position of perinuclear fat. Magnification × 170.

FIG. 36. Gelatine section of muscle from a case of muscular dystrophy with periodic paralysis to show numerous droplets of PAS-positive material situated within the muscle fibers. Magnification × 170.

FIG. 37. Perinuclear glycogen seen in a case of pseudohypertrophic muscular dystrophy. Magnification: × 570.

FIG. 38. Glycogen in muscle from a case of familial dystrophy to show that atrophy and hypertrophy do not affect the concentration of glycogen in the muscle fibers. Magnification × 170.

FIG. 39. Muscle taken from a patient with myopathy 48 hours post-mortem. Most of the glycogen has disappeared and that which remains is in the cross-striations. Magnification × 350.

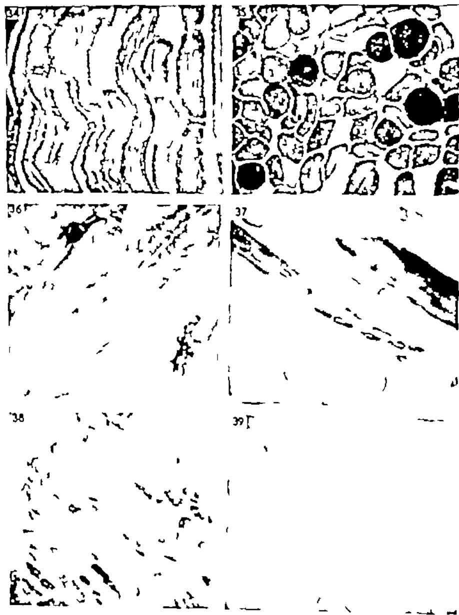


PLATE VI

far as we are aware, the present authors (Beckett and Bourne, 1958f) are the only authors to have attempted to apply this technique to the study of muscle.

In normal muscle, an irregular diffuse periodic acid-Schiff background staining is obtained The material which is stained in this is totally different in appearance from that observed in muscle fixed by a suitable method for the preservation of glycogen and so is almost certainly not this compound. One must qualify this, however by saying that just occasionally traces of material are present which might be glycogen which has not been removed during the processes of fixing and embedding

In addition both in rat and normal human muscle there are sometimes droplets of a material which gives a strikingly intense periodic acid-Schiff reaction. In pathological samples of muscle, the number of these droplets is often considerably increased The number present is not correlated in any way with clinical diagnosis, and it seems that these droplets accumulate at points of mechanical damage in the muscle fibers, e.g in areas of Nageotte's change, or in areas where the myofibrils are distorted, or again where there is vacuolation. The presence of more of these droplets in pathological samples of muscle is probably due to the fact that this muscle is more fragile than normal and would therefore be more liable to mechanical damage.

The nature of the strongly periodic acid-Schiff positive droplets is far from clear They are certainly not glycogen since their morphological appearance and distribution is totally dissimilar and they are never removed to the slightest extent by diastase. They do not represent gelatin which has become entrapped in the sections, since this material (gelatin) stains poorly with the periodic acid-Schiff technique. Further tests on the material of which the droplets are composed have shown that it is not metachromatic and does not stain either with hematoxylin and eosin, or with Schiff's reagent without prior oxidation with periodic acid. The material is not soluble in cold absolute alcohol xylol, benzene, or methyl benzoate, but it is extracted during paraffin embedding of formalin or calcium-formalin fixed material This extractability would suggest that it might be a lipid of some sort, but it is not sudanophilic and does not give a positive Schultz reaction for cholesterol In fact, [illegible] of this material remains a complete m[illegible] ough [illegible] lipid of some sort.

VIII. FAT IN SKELETAL MUSCLE

The distribution of fat in skeletal muscle is a problem which has occupied the thoughts of various workers for the last hundred years or more although from time to time confusion has crept in because of the difficulty of differentation between true fat droplets and mitochondria.

Henle in 1841 (see Holmgren, 1910) was the first to observe granules between the myofibrils which were less soluble in acetic acid than were the myofibrils themselves, but the nature of the granules which he saw is not clear from the description given. In the next half century or so the intermyofibrillar granules were the subject of investigations by various workers including Kölliker (1856) Knoll (see Schaffer 1893) and others, and it became apparent that the light density of different muscle fibers had a relationship with the amount of sarcoplasm present and also with the number of granules in it. No clear line of distinction was drawn, however between the fat droplets and mitochondria.

Most of this early work was carried out on insect muscle, because of the large size of the myofibrils in its structure, but in later investigations human material was used to quite a large extent. In 1889 Walbaum (see Bullard, 1912) studied the occurrence of fats in the skeletal muscle of 119 human bodies and found "fat" droplets in some muscles of about two-thirds of them but many of these "fat" droplets were not stainable with Sudan III In the course of his work Walbaum (see Bell 1911) also observed that the fat content of the muscles of children as studied with Sudan III was not related in any way to nutritive condition.

A few years later Schaffer (1893) who also studied human muscles (including pectoralis major and gastrocnemius) established that there were wide variations in the numbers and distribution of fat droplets in these muscles, and that there was not always a correlation between the numbers of granules and the degree of optical turbidity of muscle fibers. In addition Schaffer made one or two observations on the effect of disease on the fat content of muscle. He stated that in chronic disease the "granules" i.e the mitochondria, were converted to fat, but that few fatty droplets remained, whereas in febrile diseases there are many fatty droplets derived from the "granules."

Bell (1911) studied the effect of nutrition on the fat droplet population of animal muscle and found that starvation reduced the numbers of fat droplets present, whereas overfeeding increased it. Star

vation also tended to remove the differences in turbidity which had existed between different muscle fibers. Like Schaffer before him, Bell found that there was not any constant correlation between the degree of turbidity of muscle fibers and the numbers of fat droplets present.

Bullard (1912) continued to work on this subject and confirmed many of the earlier observations on human muscle. Using diaphragm, pectoral, and eye muscle taken at autopsy he found that fat droplets occurred constantly and abundantly When fat was absent, Bullard considered this to be a result of post mortem changes, poor nutrition, or pathological changes. He also thought that post mortem changes were responsible for the occurrence of fat in a granular form.

Bullard differentiated between fat droplets and mitochondria by observing that the latter are not dissolved by absolute alcohol and do not take up fat stains, e.g Scharlach R., readily He found no mitochondria in human muscle, a fact which he attributed to lack of fresh material. From our own experience in this matter we think that this failure to demonstrate mitochondria was probably also due in part to the very small size of these bodies in human muscle, and to the extreme difficulty of staining them differentially Both, the present authors, and Dempsey *et al* (1946) before us found that mitochondrial stains also demonstrate cross-striations with equal clarity

After the work of Bullard in 1912 there was a considerable lapse of time before any further observations were carried out on this subject, but in 1946 Dempsey *et al* carried out a very careful study on skeletal muscle of rats and monkeys using several different techniques. These included Sudan IV and Sudan Black staining associated with extraction techniques, the Smith Dietrich method for phospholipids, and polarizing and fluorescence microscopy

In the course of their work, they found that both Sudan IV and Sudan Black stained minute discrete fat droplets which were occasionally and irregularly distributed among the striations. These droplets were much more numerous in some fibers than in others, and were almost invariably located in the isotropic bands. The droplets were easily removed with acetone or alcohol at room temperature. Sudan Black also stained the cross-striations, but less intensely than the fat droplets, and the staining was much less easily removed with fat solvents In addition the cross-striations also gave a positive reaction for phospholipids with the Smith Dietrich test.

The work of Beckett and Bourne (1958f) using Sudan Black

staining has confirmed most of these previous observations. In normal human muscle fat was present in droplets of a very variable size. These were mostly distributed at random but there were a few situated at the poles of nuclei. In addition, the cross-striations were stained but with a lesser intensity as has been previously observed by Dempsey *et al* (1946) The so-called neurokeratin network of the peripheral nerves also contained Sudanophilic material

As far as we could judge there was no change in the amount of fat within the muscle fibers of any of the samples taken from cases of muscular or neuromuscular disorders, but since the fat content of normal muscle is so very variable one cannot be certain of the validity of these results.

IX. SUMMARY

1 In human skeletal muscle the succinic dehydrogenase activity is variable, depending partly upon the anatomical site of the muscle In muscular and neuromuscular disorders, there is a tendency for this activity to decrease, probably as an end result of other metabolic disorganization within the muscle fiber

In animal muscle, there is probably less variability in normal succinic dehydrogenase levels.

2 The histochemical technique for cytochrome oxidase is far from satisfactory but it seems possible that in human muscle the degree of cytochrome oxidase activity is not directly related to that for succinic dehydrogenase

3 There is a considerable overlap in substrate specificity between the broad histochemical classification of esterases into simple esterases, lipases, and cholinesterases.

It seems, that with rare exceptions, all of these esterases are totally inhibited by acetone a fixative which is frequently used in procedures for demonstrating simple esterases and lipases. However using fresh frozen sections, it has been possible to detect these enzymes in muscle fibers and in motor end plates.

4 Cholinesterase (i e eserine sensitive esterase) is present in motor end plates, in other structures of basically similar construction and also in the muscle fibers themselves.

In vertebrates other than man the specialized structures containing cholinesterase comprise (a) motor end plates of a uniform type for a given species, (b) structures, probably stretch receptors, at musculo

tendinous junctions, and (c) end plates at the poles of muscle spindles.

In man the variety of cholinesterase-containing structures is much greater and some of these structures may represent stretch receptors situated at places other than the musculo-tendinous junctions.

In the course of muscular and neuromuscular disorders, there appears to be little change in the amount of cholinesterase present in any of the definite structures in human muscle but there may be some structural disorganization. The amount of cholinesterase within the muscle fibers themselves appears to increase in degenerating fibers. In contrast to this, the motor end plates appear to survive, possibly intact, and to retain their cholinesterase activity even when the structural changes within the muscle fiber have been severe.

5 Alkaline phosphatase is normally situated in the endothelia of blood vessels of mammalian muscle, but may occasionally appear in the muscle fiber substance and in reticular like fibers in cases of human muscular and neuromuscular disorders.

6 Acid phosphatase is present in highest concentration in peripheral nerves, in adipose tissue and also in granules situated at the poles of the nuclei both of connective tissue and of the muscle fibers themselves. *A slightly lower concentration is present in nuclei, particularly those* of the connective tissue and blood vessel walls. There is some enzyme activity in the muscle fibers themselves and in the smooth muscle of blood vessels

In muscular and neuromuscular disorders acid phosphatase is present both in inflammatory cells and also in capillaries which invade the muscle fibers.

7 In normal human muscle, 5-nucleotidase activity as studied in acetone-fixed sections, is absent or is limited to peripheral nerves and to walls of arteries and veins. In normal rat muscle, an essentially similar distribution is found In some cases of muscular and neuromuscular disorders, the enzyme is present in large amounts in muscle fibers which are undergoing necrosis, in all types of inflammatory cells, and also in the walls of capillaries which invade muscle fibers. The degree of increase of enzyme activity is not correlated with the clinical diagnosis.

8 At an alkaline pH most other organic phosphates give a non-specific alkaline phosphatase reaction in acetone-fixed material It is necessary to employ fresh frozen sections in order to demonstrate ATPase Satisfactory methods for the demonstration of for instance

ADPase and creatine phosphatase, do not seem to have been worked out yet.

9 Protein bound sulfhydryl and amino groups have a similar distribution in muscular tissue, i.e. they are mainly located in the myofibrils of of the striated muscle fibers and in the smooth muscle cells of blood vessels. In addition sulfhydryl groups (or disulfide groups, according to various authors) are present in peripheral nerve fibers. The concentration of these groups in muscular tissue does not change in any of the muscular or neuromuscular disorders so far studied, even when there have been severe structural changes.

10 Oxidative-Schiff techniques probably demonstrate glycogen in suitably fixed paraffin embedded muscle, although there is some dispute on this point, due to the variable effect of diastase on the stained material. The amount of glycogen present in fibers does not appear to change in muscular or neuromuscular disorders

In gelatine sections, these techniques do not demonstrate glycogen but disclose the presence of a strongly staining material in the form of droplets which may be a lipid of some sort.

11 The fat content of human muscle is variable in the extreme, and there appears to be no change in muscular or neuromuscular disorders.

Appendix

Recent Studies on Dystrophic Muscle

Bourne and Golarz (1959 a, b) and Golarz and Bourne (in press) have carried out an extensive histochemical study of dephosphorylating enzymes in ten cases of human muscular dystrophy and three normal healthy controls. The work followed on that referred to earlier by Beckett and Bourne (1957 58) in which they noted that the connective tissue (endomysium) in muscular dystrophy actively dephosphorylated adenosine 5 mono-phosphate. Bourne and Golarz used as substrates a wide variety of esters of metabolic importance these included glucose-1 and glucose-6 phosphates, fructose 1 and -6 phosphates, hexose d phosphate and phosphoglyceric acid, high energy phosphates such as ADP ATP uridine triphosphate, inosine triphosphate acetyl phosphate and creatine phosphate and phosphorylated co-enzymes such as riboflavin-5 phosphate pyridoxal phosphate thiamine pyrophosphate and di- and tri-phosphopyridine nucleotides. It was found that the connective tissue of dystrophic muscles did not dephosphorylate the glycolytic intermediates but that it actively hydrolyzed all the high energy phosphates with the exception of creatine phosphate which was only slightly hydrolyzed. Of the co-enzymes, riboflavin and pyridoxal phosphates, were unaffected, but in five out of ten cases the thiamine pyrophosphate was very actively hydrolyzed by the connective tissue The most constant dephosphorylating activity was for TPN and particularly for DPN

Another factor which was of interest was that in dystrophic muscle there was an accumulation of phosphatases capable of hydrolyzing all the substrates listed above

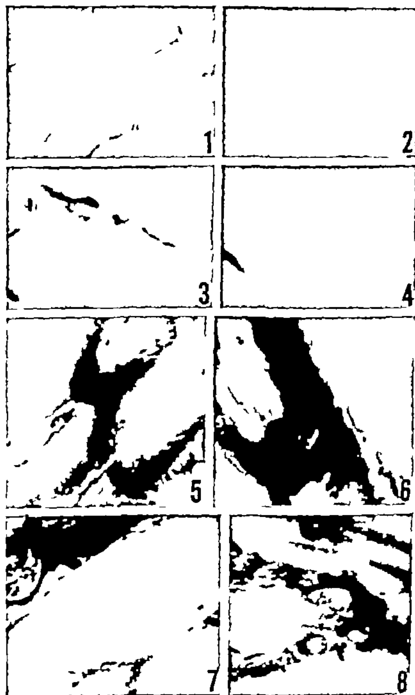


PLATE VII

on what appeared to b the surface of the sarcolemma. Such an accumulation of phosphatases was not found in normal human muscle.

The possible significance of all these results was discussed by Bourne and Golarz (1959a) in an article in Nature (1959) They came to the conclusion that there were three possible interpretations of their findings.

1) Although these metabolically important phosphate compounds are synthesized by muscle there may be some leakage in normal metabolism much as Vitamin A leaks away from the retina in the retinene cycle. Replacement of this leakage by transport from the vascular and lymphatic system If there is inadequate synthesis by the muscle itself would be prevented by connective tissue containing the appropriate phosphorolytic enzymes.

2) The fundamental defect may still be in the membrane of the muscle fiber and the diffusion of phosphate esters out into the surrounding milieu may result in adaptive synthesis of enzymes to hydrolyze them in the connective tissue.

3) That the excessive production of these hydrolytic enzymes by the connective tissue leads to their diffusion to, adsorption on to, and eventual penetration of the muscle fiber membrane resulting in complete blocking of the normal metabolism of the fiber

Of these alternatives "2" requires further investigation and of "1" and "3" the latter appears to be more in keeping with current views of muscle metabolism although the penetration of the sarcolemma by hydrolytic enzymes has not been established. However it has been shown that a wide range of phosphatases ar in fact associated with the sarcolemma in dystrophic muscle fibers and it does not seem improbable that these enzymes would affect the functioning and possibly th structural integrity of the sarcolemma. It may be that the fundamental defect in muscular dystrophy lies primarily in the endomysium rather than in the muscle fib r

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FIG. 1 Human muscle Normal (fixed in acetone) Substrate adenosine triphosphate (ATP) pH 9.0 Negative reaction.

FIG. 2. Human muscle Normal (fixed in acetone) Substrate diphosphopyridine nucleotide pH 9.0. Negative reaction.

FIG 3. Human muscle. Dystrophic Substrate glucose-6-phosphate. pH 9.0. Reaction only in capillaries.

FIG. 4 Human muscle. Dystrophic. Substrate fructose-6-phosphate. pH 9 0. Reaction only in some of capillaries.

FIG. 5. Human muscle Dystrophic. Substrate adenosine triphosphate (ATP) pH 9 0 Intense positive reaction in all elements of proliferating connective tissue. Muscle fibers negative (heat-stable ATPase)

FIG. 6. Human muscle Dystrophic Substrate adenosine diphosphate (ADP) pH 9 0 Reaction similar to ATP

FIG. 7 Human muscle Dystrophic Substrate diphospho-pyridine nucleotide DPN) pH 9 0 I tense reaction in all elem nts of proliferating connective tissue Muscle fibers negative

FIG. 8. Human muscle. Dystrophic Substrate triphosphopyridine nucleotide TPN) pH 9 0 Intens reaction in all elements of connective tissue Muscle fibers negative

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CHAPTER X

Myopathy the Pathological Changes in Intrinsic Diseases of Muscles

F D Bosanquet P M Daniel, and H. B Parry

I. INTRODUCTION

The greater part of the human body is composed of muscle tissue and imperfect working of this tissue causes the most severe and disastrous effect on the day to-day life of the patient, yet we know less about the pathology of the muscles than about that of any other organs. However there appears to be a growing interest in the subject, for within the last few years several monographs have appeared (Adams *et al.*, 1953 Greenfield *et al.*, 1957 Walton and Adams, 1958) which supplement earlier work (Erb 1891 Pick, 1900 Durante 1902 Steinert, 1909

Marinesco, 1910 Jendrassik, 1911, Meyenburg 1929 Wohlfahrt and Wohlfart, 1935 Slauck, 1936), though our knowledge of this obscure field still remains slight. A number of studies, though concerned more with the clinical features than with the pathological changes in myopathy should be consulted by all those interested in the subject (Meryon 1852 1864 Duchenne, 1868, 1872 Gowers, 1879 1902 Erb, 1884 1894 Batten 1910a, b Spiller 1913 Bing, 1926 Rouquès, 1931 Curschmann, 1936 Hurwitz, 1936 Sjövall, 1936 Wilson, 1940 Bell, 1943 1947 Shank *et al* 1944 Levison, 1951, Welander 1951 Becker 1953 Schoen and Tischendorf, 1954 Walton and Nattrass, 1954 Bouman, 1955)

Genetic studies have thrown considerable light on the problem of myopathy especially those of Tyler in America (Tyler and Wintrobe, 1950 Tyler and Stephens, 1950 1951 Stephens and Tyler 1951 Tyler 1951 1954) and Stevenson in Ireland (Stevenson, 1953 1955 Stevenson *et al*, 1955)

In this paper "myopathy" is used to describe any disease which is primary to muscle and not of neural origin. Knowledge of the pathology of muscle is at present elementary and it is possible that various conditions which now appear to be primary muscle diseases will be found to be secondary to disease elsewhere

II. VARIETIES OF CHANGE IN PATHOLOGICAL MUSCLE

A. CHANGES IN MUSCLE FIBERS

Normal skeletal muscle fibers are polyhedral in cross section and appear of relatively equal diameter They have peripherally situated subsarcolemmal nuclei The extrinsic ocular muscles, however have in general small and rounded muscle fibers with some central nuclei occasional fibers of large diameter are present (Cooper *et al* 1955) Occasional small muscle fibers, which have no pathological significance are seen in all muscles they are the extracapsular parts of the intrafusal muscle fibers of the muscle spindles (see Chapter XI Vol. I by Cooper on muscle spindles)

Abnormal muscle fibers are usually rounded in cross section and may be either larger (hypertrophied) or smaller (atrophied) than normal. They may also show a variety of changes in the cytoplasm while the nuclei may be increased in number or unusual in appearance or centrally placed within the muscle fiber An abnormally great variation in

the caliber of the muscle fibers together with obviously atrophied fibers is usually the most striking change in a severely diseased muscle, and the distribution of such fibers may give the clue to the disease. Atrophying muscle fibers may simply lose substance so that they appear as merely thin normal fibers (Fig 2) or the cytoplasm may show degenerative changes, such as breaking up into short segments The cytoplasm may disappear from the sarcolemmal tube, which is left empty and shrunken except for a row of nuclei. The sarcolemma may disappear so that isolated nuclei or small masses of nuclei alone remain.

Hypertrophy of the muscle fibers is less easy to recognize with certainty than atrophy since normal muscle fibers may appear enlarged if adjacent ones are atrophied when all the fibers in a field are enlarged, hypertrophy may also be difficult to recognize Genuinely enlarged fibers may be otherwise normal, as in compensatory hypertrophy and some dystrophies, but enlargement of muscle fibers is often only part of a degenerative change as for example when a segment of a muscle fiber is swollen and the sarcoplasm is glassy and without striations (Fig 20) Such swollen fibers commonly fragment, forming at first isolated segments, with an empty sarcolemmal sheath stretched between them, (Fig 13) and then later becoming irregular masses of sarcoplasm (Fig 17) with either peripheral nuclei or nuclei in their substance.

Measurement of muscle fiber diameter requires accurate transverse sections. As muscle fiber sizes vary greatly with age, nutritional status (Hammond 1932) and in different skeletal muscles, it is essential to know the normal variation in fiber size, for the particular muscle, before interpreting any changes as being pathological. Data on normal human fiber sizes are scanty but some information is available (Halban, 1894 Feinstein *et al.*, 1955 Sissons, 1955 Greenfield *et al.*, 1957) In muscles concerned with delicate movements e g the lumbricals, the fiber sizes are small 18μ (Feinstein *et al.*, 1955) while in large muscles concerned with coarse movements, e.g tibialis anterior the fibers are large 56μ (Feinstein *et al.*, 1955) However the range of size is considerable, for Greenfield *et al.* (1957) in specimens of vastus lateralis from normal volunteers, found a range of fiber sizes from 25 to 90μ. We would regard any considerable number of fibers of over 100μ as beyond the range of normal.

In the present state of knowledge the actual measurement of muscle fibers, particularly in biopsy material, is of limited value

The cytoplasm of muscle fibers may undergo a number of pathological changes. The striations may be lost and the cytoplasm may become hyaline (Fig 20) The cytoplasm may show fine granules (granular degeneration, Fig 16) or larger floccules (floccular degeneration Figs. 18, 20) becoming at the same time intensely eosinophilic. Vacuolation may occur, especially in much swollen fibers. Floccular degeneration may be segmental and is accompanied by an ingrowth of nuclei, some of which are sarcolemmal nuclei and some invading phagocytes. Portions of the sarcolemmal tubes may thus come to be filled with nuclei and the remains of sarcoplasm (Figs. 14 21)

A pathological basophilia of the cytoplasm (sometimes difficult to distinguish from artifactual staining) also occurs. Isolated necrotic muscle fibers are often intensely basophilic, staining purple throughout with hematoxylin. A milder degree of basophilia is seen in thin regenerating fibers (Fig 19) and in the multinucleated sarcoplasmal masses ("muscle buds") containing numbers of vesicular nuclei which are also found in regenerating muscle, although it is not certain that the presence of these "muscle buds" always denotes regeneration.

An increase, or at least an apparent increase, in the number of sarcolemmal nuclei is seen in almost every abnormal muscle and is commonly the first change discernable. The nuclei tend to be more rounded and vesicular than normal and prominent nucleoli may appear (Fig 16) The nuclei may form peripheral chains (Fig 15) but often they invade the sarcoplasm, becoming centrally placed, in rows or long chains, especially in dystrophia myotonica (Figs. 8–11) Centrally placed nuclei in adult muscles, other than the extrinsic eye muscles, are usually abnormal. Nuclei of abnormal muscle fibers may be pyknotic and densely staining Large clumps of darkly staining nuclei may be seen in and among atrophic fibers (Figs. 2 6)

Other unusual appearances seen in muscle fibers are ringed fibers and split fibers. Ringed fibers ("ringbinden" or spiral annulets) consist of muscle fibers encircled by cross-striated muscle fibrils, as a finger is encircled by a ring, lying within the sarcolemmal sheath. The arrangement of these cross-striated encircling fibrils is thought to be spiral Greenfield *et al* (1957) Their significance is unknown but they may increase with age, though we have seen them in muscles, including the eye muscles (Daniel 1946) of normal people of all ages. Heidenhain (1918) thought that they were specifically associated with dystrophia myotonica but though common in this disease, they cannot

be regarded as diagnostic (Wohlfart, 1951) Longitudinal splitting of muscle fibers is seen only in pathological muscles the complete muscle fiber and the sarcolemma split, and in longitudinal section, the actual division of a fiber may be seen, as in cardiac muscle. In cross section, the appearance is of two fibers lying within a single endomyosial sheath.

B Changes in the Interstitial Tissue

The interstitial tissue shows changes in many varieties of muscle disease. The changes, like those in the muscle fiber are not specific for any particular disease but appear rather to reflect the rapidity or chronicity of the change in the muscle tissue. Interstitial fat is in creased in most cases of prolonged muscle wasting but it is most marked in the Duchenne type of muscular dystrophy (Figs. 1 2) and in thyrotoxic myopathy Normal muscle contains virtually no fat between the muscle fibers (Chapter 2) but as muscle fibers are lost rows of fat cells may be found separating the remaining fibers. Thus isolated groups of fibers surrounded by collagen (thickened endomy sium) are found lying in adipose tissue which occupies the site of the original muscle (Fig 1) In this adipose tissue lie the structures which have not degenerated the blood vessels, nerves and muscle spindles.

Collagen also is increased in almost all chronic myopathies. The relative amount of adipose and fibrous tissue varies from case to case but in general collagen is the main constituent increased after prolonged denervation and also in the very chronic Landouzy Dejerine type of muscular dystrophy fibrous tissue may form the main bulk of what was a muscle and the original vascular bed, nerves, and muscle spindles lie embedded in collagen (Figs. 3–6) It was formerly thought that muscle fibers were actually transformed into either fat or collagen, but this view is not now accepted and though the process is not fully understood it is generally believed that there is replacement rather than meta plasia. The well marked fibrous capsule of normal muscle spindles and the small intrafusal muscle fibers, which often have central nuclei have been erroneously described and illustrated as pathological structures (Ingram and Stewart, 1934 Bevans, 1945)

An increase of cells in the interstitial tissue (Figs. 12, 19) may be found in any active muscle disease In most cases, mononuclear cells predominate while phagocytes, lymphocytes, plasma cells, and fibroblasts, as well as free sarcolemmal nuclei from disintegrated fibers may all be present. Both neutrophilic and eosinophilic polymorpho-

nuclear cells may be present, but they are seldom the predominating cell it should be mentioned however that a noticeable number of polymorphonuclear cells may be present even in a dystrophic muscle of many years standing

The endothelial nuclei of the capillaries are often enlarged and may on occasion be difficult to distinguish from sarcolemmal nuclei. A cellular reaction may be found in an actively degenerating muscle in muscular dystrophy yet, in general, the infiltration is greater in polymyositis. *It is to be noted that a quite marked cellular reaction* may also be present in a muscle after rapid denervation (as after poliomyelitis) and even in the muscles of bedridden patients.

Lymphorrhages are focal collections of small round cells (lymphocytes) lying between muscle fibers which appear normal, and are seen classically in myasthenia gravis, but they may also be found in poly myositis, rheumatoid conditions, and in the ocular muscles in ex ophthalmic ophthalmoplegia, where the foci of round cells can reach an enormous size.

C. Artifactual Appearances

Variation in the staining of fibers may cause considerable difficulty in sections of muscle tissue. Whether artifactual or due to genuine differences in the fibers, the following variations are found in sections of normal muscle. The staining may simulate either basophilia or eosinophilia and, furthermore the same fibers may show an abnormal staining in several sections. The artifactual nature of the staining is fairly easily recognized if it occurs in a block of fibers or perhaps at one side of the section When, however scattered fibers stain abnormally they may be difficult to assess and the only safe rule is to ignore all variations in staining unless there are other unequivocal pathological changes. This also applies to an apparent loss of cross striations, a common finding in normal muscle fibers, and one which should not be accepted without other and certain, evidence of abnormality Tissue which has been fixed in extreme contraction, or too rapidly (notably with osmic acid or alcohol) may show fragmentation of the myofibrils into irregular darkly staining bands (Nageotte s contraction bands) or into longer segments with condensed contraction bands at the ends. Bad cutting and brittle material may also cause multiple fragmenta tion but this is usually obvious. *Longitudinal splitting* within the sarcolemmal sheath vacuolation, and shrinkage of the fibers away from

the endomysium are difficult to assess as they often accompany pathological changes (Figs. 11 14) though they are often artifactual and should therefore be discounted. The scattered dropping out of fibers, sometimes seen in transverse sections, may simulate early fatty infiltration.

III. DISTINCTION BETWEEN NEUROPATHIC AND MYOPATHIC CHANGES IN MUSCLE

Degeneration of a motor nerve is, as is well known, followed by degeneration of the muscle fibers supplied (Fig 7) It is therefore necessary in all cases of muscular wasting to distinguish between lesions which are of neuropathic origin and those which are myopathic. This may be simple or very difficult.

The histological characteristics of a *neuropathic* muscle lesion are dependent upon the fact that only those muscle fibers atrophy which have lost their nerve supply and secondly that compared with myopathic conditions, the lesion is a relatively pure atrophy with little other change. Thus the typical histological finding is groups of small muscle fibers lying beside normal sized ones. This is best seen in a transverse section where the atrophic fibers appear as small scattered groups (i e. motor units) or there may be larger groups consisting of whole fasciculi (Fig 7) or again an entire muscle may be atrophied as after poliomyelitis. The atrophy is generally quite obvious, the affected muscle fibers often being only 5 or 10μ across with an increase in nuclei while the unaffected fibers apart from sometimes being rounded, show no abnormality the staining reaction of all fibers is usually normal. The nuclei of the small fibers lie close together on account of the shrinkage of the cytoplasm. When degeneration is far enough advanced, nuclei within a sarcolemmal sheath without any sarcoplasm and free sarcolemmal nuclei are present among the atrophic fibers these nuclei tend to be hyperchromatic. The unaffected muscle fibers remain normal, apart from a tendency to become rounded and possibly to develop some compensatory hypertrophy Little is known as to the extent of this hypertrophy but it is seldom striking and varies a good deal from case to case In time, the collagen around groups of atrophied muscle fibers increases, and may in fact demarcate "islands" of such fibers this is in contrast to myopathic lesions where the collagen tends to encase each individual fiber

In longitudinal section groups of long very thin muscle fibers, and also fibers represented only by a nucleated sarcolemmal sheath are

found beside normal fibers. Short segments of fibers which have fragmented are also seen, often with cross striations still preserved, and sometimes capped at the ends with hyperchromatic nuclei. The number of nuclei in the degenerating bundles of muscle fibers appears to be greatly increased, and there are frequently clumps of massed nuclei on and between the fibers. It is uncertain whether this apparent increase is due entirely to the approximation of nuclei from loss of the intervening sarcoplasm. There is remarkably little interstitial infiltration with cells, except occasionally when a large amount of muscle tissue is degenerating An increase of adipose tissue, although often present, is seldom conspicuous. Degenerating muscle fibers may have central nuclei, but swollen segments, floccular degeneration and phagocytosis of the muscle fibers, though they may be present, are rare.

This is the characteristic picture of a muscle with a partial denervation of some standing The time taken for a diagnosable lesion to develop varies much, but is at least several weeks, and often, in slowly progressive diseases many months. Adams *et al* (1953) after experimental nerve section in animals, found an increase of nuclei within 3 weeks and just visible atrophy in a month, but with unexplained variation in the rate of degeneration even within a single muscle. In humans, after acute denervation, there may be obvious degeneration in 4–6 weeks, but in chronic diseases, it may not be discernable until long after clinical weakness is apparent (9 months or more) No signs of regeneration of muscle fibers occur after complete nerve section.

Peripheral nerves included in a muscle biopsy may show evidence of degeneration such as fibrosis and loss of myelinated fibers fat staining of frozen sections may show myelin sheaths breaking down in the more acute cases, and silver staining may show degenerating axons. Intravital staining of nerve fibers with methylene blue may also demonstrate abnormal nerve fibers and end plates (Coërs and Woolf, 1959) In practice however only a minority of routine biopsies afford this information partly because nerves to muscles are mixed (motor and sensory) and partly because histological changes must be unequivocal to be significant. Greenfield *et al* (1957) in a series of 121 biopsies found significant changes in the nerves of only 2. We have found the presence of obviously normal nerve trunks more valuable in excluding a neural lesion than the converse

In a myopathic lesion the distinction between normal and abnormal muscle fibers is much less sharp, as, in contrast with a partially

denervated muscle, each muscle fiber is affected as a single unit although the whole muscle is abnormal. Therefore atrophic fibers are not found in localized groups as after denervation but muscle fibers of all sizes may be intermixed while few fibers are normal Often many or all of the muscle fibers are rounded in cross section and enlargement of fibers is often conspicuous (Fig 3) this enlargement, either absolute or relative, frequently persists until a late stage in the disease Degenerative changes other than atrophy are more common in myopathic than in neuropathic muscles the muscle fibers show changes in staining reaction and loss of cross striations more frequently Floccular degeneration of part of a fiber and phagocytosis of the sarcoplasm with segmental disruption of the fiber are seen more often, particularly in the more acute myopathies. In any actively progressing lesion an interstitial infiltration of cells may be found, while an increase in endomysial collagen and infiltration of fat between the individual fibers is a more characteristic feature of a myopathy than a neuropathy

These are the distinguishing features in typical, fairly well advanced cases of denervation and myopathy but there are many modifying factors which may make it difficult or impossible to distinguish between the two in a particular biopsy e.g when the condition is too far advanced or not advanced enough The earliest change after denervation is an apparent increase in the number of sarcolemmal nuclei which at this stage are more vesicular than usual this change is not specific and may be seen in any abnormal muscle. In the final stages of both a neuropathic atrophy and a myopathy a few muscle fibers only may remain, surrounded by collagen or adipose tissue it may not be clear by which process the lesion originated The characteristic pattern of neural atrophy is not present after complete denervation, as all the fibers show rounding and shrinkage and, furthermore a few scattered fibers may remain larger than the others for a considerable time Segmental degeneration may be present in a neuropathy as well as in a myopathy

It must be emphasized that the histological changes in muscle fibers are not specific to a particular etiology and that the histological appearance often shows little correlation with the clinical state. Age normal variations, and disuse must all be considered and it should be remembered that active degeneration may be found in muscle from patients who have been in bed awhile

It is therefore obvious that a positive diagnosis can only be made in a proportion of muscle biopsies. This proportion is increased if the

muscle selected shows well marked atrophy clinically if a good sized specimen is taken in such a way that accurate transverse and longitudinal sections may be made, and also by careful processing

IV THE MUSCULAR DYSTROPHIES

Muscular dystrophy is the name given to a number of syndromes which have one feature in common a progressive weakness of the voluntary (and sometimes cardiac) muscles, without evidence of inflammation in the muscles or of involvement of the nervous system. The muscular dystrophies are extremely chronic, usually progressing slowly for many years, and the patients often have a family history of similar disease.

It is not clear whether muscular dystrophy is really one disease, as Erb (1891 1894) thought when he gave the name dystrophia muscularis progressiva, or a number of related diseases However since the clinically different syndromes seem to be constantly inherited (see Section I) it appears that there are some genuine differences. Unfortunately a number of cases are met with which do not fit clearly into the classic types but show some features of each.

The cause of muscular dystrophy of any type is quite unknown, but the diseases are thought to be essentially "degenerative" and possibly due to inborn errors of metabolism or endocrine disturbances. Much work has been done on the biochemical aspects of these conditions, but this has thrown little light on the pathological changes in the muscles. An increase in creatine excretion and a decrease in creatinine excretion is regularly found but this abnormality occurs in any condition in which muscle tissue is being broken down.

Adams *et al* (1953) suggest that the lack of evidence of regeneration is the fundamental characteristic of muscular dystrophies. Walton and Adams (1956) however consider that regeneration of muscle fibers may occur Little work has been done on the innervation of dystrophic muscles.

A. Duchenne Type of Muscular Dystrophy

Duchenne type of muscular dystrophy characteristically begins in boys under 5 years of age, being inherited as a sex linked recessive (Tyler 1950 Stevenson, 1953) The muscles of the pelvic girdle and

Synonyms pseudohypertrophic paralysis, Duchenne (1868); pseudohypertrophic muscular dystrophy progressive muscular dystrophy of childhood, Tyler and Wintrobe (1950) Duchenne type rapidly progressive muscular dystrophy of young boys, Stevenson (1953) and severe generalized familial muscular dystrophy Adams *et al* (1953)

the quadriceps femoris are usually involved first while enlargement of the calf muscles is very characteristic. The condition is almost invariably progressive and most cases die of intercurrent disease (usually pulmonary infection as a result of weakness of the respiratory muscles) before they are 20 The facial muscles may or may not be involved

The histological appearances vary greatly depending on the stage that the disease has reached and the degree of involvement of the particular muscle examined. Examination of many muscles obtained at autopsy gives the only adequate idea of the extent of the disease, and of the severity of the pathological changes in the muscles thus, biopsy specimens may be misleading

A muscle taken from an early case may show little change beyond rounding of the muscle fibers and a slight increase in the sarcolemmal nuclei There may however be occasional fibers of small diameter and also a few fibers of abnormally large diameter over 100μ, but otherwise normal. The cause of the enlargement of the whole muscle is not clear since often so few of the individual muscle fibers are enlarged but there may already be an increase in the amount of the interstitial fat Small focal areas of necrosis may be seen in a muscle fiber but this is only an occasional finding

When the terminal stage of the disease has been reached and at necropsy the skin is removed an astonishing sight meets the eyes The bulk of the musculature of the body sometimes without much loss of size, appears to have been sculptured in fat. The architecture of the various muscles is quite clear but of ordinary brown muscle tissue, hardly a trace is seen. The intercostal muscles, the diaphragm the tongue and muscles of mastication, the extrinsic eye muscles, and perhaps the lumbricals of hands and feet alone probably resemble normal muscles, though they are very pale

Microscopically a cross section of one of these muscles shows that the muscle tissue has disappeared to an astounding degree. Only occasional rounded muscle fibers are seen (Fig 1) some of which may be unusually large and others small Whole microscopic fields may have to be searched before a single muscle fiber is found in the expanses of fibro-fatty tissue in which the muscle spindles stand out clearly for the intrafusal muscle fibers of these organs seem to be relatively well preserved, although we know little of their functional state or innervation

Clearly there are very many intermediate states to be seen between











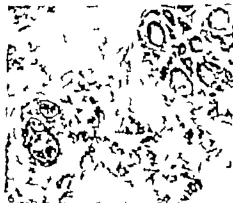


PLATE I

the stage in the disease when there is a little simple rounding of muscle fibers with slight variations in fiber size and the terminal stage of the disease when virtually no muscle fibers are to be seen.

B Facio-scapulo-humeral Type of Muscular Dystrophy

The onset of this variety of muscular dystrophy is usually later than it is in that described above as the Duchenne type, though it may be diagnosed at any age Males and females are about equally affected Tyler considers that the disease is inherited as a simple somatic Medelian dominant (Tyler 1951) others believe that a family history is not invariable. Weakness and wasting of the shoulder girdle, especially the lower fibers of the pectoralis major and trapezius, is

[1] Synonyms atrophic progressive myopathy of Landouzy-Dejerine (1885a) Juvenile form of progressive muscular atrophy Erb (1884 1894) progressive muscular dystrophy facio-scapulo-humeral type Tyler and Wintrobe (1950) autosomal limb-girdle muscular dystrophy Stevenson (1953) and mild restricted muscular dystrophy Adams *et al* (1953)

Fig. 1 Duchenne type of muscular dystrophy from a boy aged 14 Scattered groups of rounded muscle fibers of all sizes embedded in adipose tissue. (Transverse section, soleus, iron hematoxylin and van Gieson. Magnification × 65)

Fig. 2. Same case as Fig 1 Longitudinal section of a small bundle of atrophic muscle fibers in adipose tissue showing well preserved cross striations, clumps of sarcolemmal nuclei, and an increase of collagen around the muscle fibers. (Plantaris, iron hematoxylin and van Gieson. Magnification × 350.)

Fig. 3. Facio-scapulo-humeral muscular dystrophy in a man aged 21 with a family history of the disease. Severe atrophy on the left; tiny muscle fibers are seen and also sarcolemmal nuclei in empty sheats, all embedded in dense fibrous tissue. On the right, very large rounded muscle fibers. One fiber (arrow) undergoing active phagocytosis. There is an increase in endomysial collagen. Normal-looking muscle spindle in center with nerve above. (Transverse section, vastus medialis, hematoxylin and eosin. Magnification × 65.)

Fig. 4 Same case as Fig. 3 Another field of the same muscle showing more severe atrophy and fatty infiltration. Scattered, large muscle fibers and fibers in various stages of atrophy remain. There are two normal-looking nerve trunks. (Transverse section, hematoxylin and eosin. Magnification × 65.)

Fig. 5. Facio-scapulo-humeral muscular dystrophy: from girl aged 15, with family history Showing muscle virtually replaced by collagen. Between the two muscle spindles (right and left) one recognizable muscle fiber and the remains of many atrophic fibers are seen. (Transverse section, biceps brachii, iron hematoxylin and van Gieson. Magnification × 140.)

Fig. 6. Adjacent field to Fig 5. Severe atrophy A single bundle of rounded muscle fibers of very unequal size on the right. Normal looking muscle spindle on the left, with sarcolemmal nuclei in intervening dense fibrous tissue. (Transverse section, biceps brachii, iron hematoxylin and van Gieson. Magnification × 140)

often the earliest feature of the disease, followed by involvement of the muscles of facial expression though Batten (1910a) points out that these latter may be affected at birth. There is seldom enlargement of the affected muscles in the facio-scapulo-humeral type of dystrophy which thus differs markedly from the Duchenne type, where hypertrophy of the leg muscles is common and where the disease is virtually confined to males (entirely confined to boys according to Stevenson, 1953) The inheritance is very different (Tyler 1951)

Facio-scapulo-humeral muscular dystrophy is an astonishingly chronic disease. Landouzy and Dejerine (1885b) described an autopsy on a case whose symptoms began, in the face, at the age of 3 years and who died of phthisis at the age of 24 at a relatively early stage of the muscle disease The central and peripheral nervous systems were normal. They described a simple atrophy of muscle fibers which retained their striations and showed no increase in nuclei. The connective tissue was increased and there was some increase in fat. In 1886, these authors reported another autopsy (scapulo-humeral type) in a man of 66, who had suffered from the disease for over forty years. There was an intact nervous system with atrophy of the limb-girdle muscles. However in this case, they reported a marked increase of the sarcolemmal nuclei and hypertrophy of muscle fibers in certain muscles. In the very atrophied muscles, they found an increase of interstitial fat without much sclerosis. The third classical case upon whom an autopsy was performed was a patient described by Landouzy and Dejerine in 1884 and 1885a, who did not die until 1902 aged 45 having been studied by these acute observers for nearly 30 years. Landouzy and Lortat Jacob (1909) performed a very careful dissection of this case. They found that the muscles of the limbs were much atrophied, pale, yellow-gray in color and very fibrosed. Some were mere rigid cords. The muscles of the thenar eminence were degenerated and resembled tendons, although the hypothenar and lumbrical muscles were not so badly degenerated. The muscles of the neck and trunk were also much atrophied The supra and infraspinati and subscapulares appeared normal in color Only a trace of the facial muscles was found.

Histologically they found that, in the case of the most severely affected muscles, they could not be sure that striations were present, the muscle fibers appearing granular with only doubtful striations. In less severely affected muscles, they found proliferation of nuclei in chains. The interstitial fibrous tissue was much increased and they

thought the muscle fibers were compressed. There was much adipose tissue in the interstices. The central nervous system and peripheral nerves did not show degeneration. We have been unable to find any other autopsy reports in the literature in which a systematic study of the muscles has been made, although Denny Brown performed an autopsy on a case (personal communication) and examined some of the muscles. This case is referred to in Adams *et al.* (1953)

In general, in this disease the histological appearances show the changes of myopathy That is to say, the atrophic muscle fibers are scattered at random, or if they are in groups, these are irregular in contrast to a neural degeneration. Muscle fibers of large diameter some of which are always present except in the very last stages of the disease, are even more irregularly scattered All the muscle fibers tend to be rounded when seen in cross section (Figs. 3 4 and 6) The interstitial collagen increases around individual muscle fibers until, in time, most of the shrunken muscle mass is replaced by connective tissue in which the remaining muscle fibers lie either singly (Fig 5) or in small groups (Fig 6) This mass of connective tissue contains also the vascular bed of the replaced muscle, the nerve trunks, and the muscle spindles, now very prominent owing to the disappearance of so many extrafusal muscle fibers (Figs. 3 5 and 6)

The great increase in the collagenous tissue has been stressed because in this type of muscular dystrophy it is very characteristic. In this condition, there is always some, and often much increase in the adipose tissue of the affected muscles, but the extreme degree of fatty change such as is seen in the Duchenne type of dystrophy is not so striking a feature of this disease.

The earliest evidence of the disease is a rounding of the muscle fibers and irregularity of caliber with little if any increase in interstitial tissue. Both large and small fibers are seen at an early stage. The large muscle fibers (over 100μ) may be homogenous and swollen over short distances of their length. These are presumably undergoing some form of degeneration. However fibers of well over 100μ diameter are seen which are well striated and apart from their increased girth show no abnormality The significance of these large normal looking muscle fibers is not clear but it seems possible that, for some time, a work hypertrophy might occur in some fibers of a muscle which is much used. Scattered foci of necrosis may be seen in some muscle fibers, and an increase of sarcolemmal nuclei in others granular degeneration

and phagocytosis (Figs. 3 21) also occur during the early stages of the disease, and during this phase there may be some interstitial infiltration with cells, including polymorphs. These changes are found in any actively degenerating muscle and in dystrophic muscle may be present many years after the onset of the disease. The differential diagnosis from mild polymyositis may be difficult. The vessels and nerves in these cases show no pathological change, as far as is known, other than those secondary to loss of muscle fibers.

C. Dystrophia Myotonica[1]

The characteristic feature of this disease is muscular weakness accompanied by myotonia. The onset is seen most commonly in adolescence or early adult life, but it may be at any age. The disease is usually inherited as a dominant Mendelian characteristic, affecting both sexes (*Maas, 1937 Ravin and Waring 1939 Thomasen, 1946*) Weakness and myotonia are followed, after a varying period of years, by atrophy of the affected muscles.

This disease differs from the other muscular dystrophies in a number of ways. It is genetically distinct. The age of onset tends to be later and it seems probable that a number of distal myopathies of late onset are in fact examples of this disease. The distal muscles, particularly of the hands and forearms, are affected first, together with those of the face (ptosis being common) and the sternomastoids later there may be dysphagia and dysarthria, which are rare in other dystrophies. Myotonia usually precedes the weakness though it may be overlooked. Maas and Paterson (1947) believe dystrophia myotonica to be a generalized disease certainly it is accompanied by endocrine disorders. Premature baldness and testicular atrophy occur in a high proportion of cases. Thyroid or pituitary deficiency is suggested by a low basal metabolic rate and enophthalmos, while a low blood sugar curve is occasionally found. Blood cholesterol has been normal where recorded. General asthenia, suggesting adrenal deficiency is the rule, but serum sodium and potassium level has been normal in the few cases in which it has been recorded. Hypoparathyroidism, once suggested as a cause of the disease, has not been substantiated and blood calcium is normal. Acrocyanosis is often present. Premature cataract is very frequent [nearly 90% of Thomasen s (1948) cases] as was first noted by Greenfield (1911) This may be found as the only abnormal feature in the

[1] Synonyms myotonic dystrophy myotonia atrophica.

relatives of a patient suffering from the overt disease. Hormonal studies have not contributed much so far though Liversedge and Newman (1956) have found that cortisone and corticotropin reduce the duration of the myotonia. Gonadotropic hormones are decreased when there is clinical gonadal atrophy Creatinuria is usually slight, corresponding to the slow rate of muscular atrophy

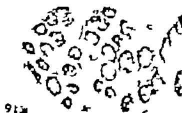
Myotonia, a hallmark of the disease, is usually found in the hands and forearms, though it may also be present in muscles which are not weak or wasted for instance, it is common in the tongue, which is rarely wasted. Myotonia is made worse by cold or fatigue and may in fact only be elicited after cooling The electromyogram is characteristic, consisting of prolonged bursts of potentials during and after a single contraction. Atrophy may be long delayed however in time the weak muscles become atrophied and are then paler macroscopically than normal muscles.

Microscopically the changes, once established, are essentially those of other chronic myopathies, that is to say rounding of the muscle fibers in cross section with atrophy of fibers singly or in groups, in either case forming an irregular pattern when seen in cross section (Figs. 8, 9) In addition to the small atrophied fibers, large fibers (100 to 150μ in diameter) may be present, and indeed may be common in the early stages. These may appear normal apart from their size or may have central nuclei (Fig 8) Any of the various types of muscle fiber degeneration may be present, but in so chronic a disease atrophy with empty sarcolemmal tubes is usually more prominent than acute degeneration. Splitting of muscle fibers is said to be rare (Adams *et al.*, 1953) but we have seen it in several cases and Wohlfart (1951) speaks as though it is common In fact, he describes (and illustrates) capillaries apparently within the muscle fibers as a result of splitting There is a generalized increase in cellularity but on the whole, interstitial infiltration of cells is slight The degree of increase of collagen and fat corresponds with the amount of muscle fiber atrophy and until the atrophy is severe the interstitial overgrowth is only moderate in amount. A great excess of fat between the muscle fibers is seldom seen except in the final stages of a severe atrophy

A striking feature histologically and one much stressed by writers on the subject is the presence of long rows of central nuclei (Figs. 10 11) within the muscle fibers (Adie and Greenfield, 1923) These nuclei are more rounded than usual and often contain prominent nucleoli









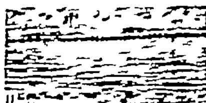






Plate II

Such rows of nuclei in otherwise normal looking muscle fibers have been called diagnostic (Adams *et al.* 1953). This view should be accepted with caution, particularly in biopsy specimens, as rows of central nuclei of considerable length may be found in other conditions but it is true that really long chains (10–20 or more) of central nuclei are often a striking feature in dystrophia myotonica and that they may also be present in clinically unaffected muscles in cases of this disease. The peripheral muscle nuclei may also be arranged in chains, but this is not unusual in other conditions (Fig. 15). Wohlfart (1951) has observed that a thick peripheral layer of clear sarcoplasm without myofibrils is present in many muscle fibers, due to loss of myofibrils without a corresponding shrinkage of the sarcoplasm, and believes that this is characteristic of the disease. He also agrees with others (e.g. Heidenhain 1918) that "ringbinden" are common, though he is careful to point out that these may be found in normal muscles.

Owing to the extreme sensitivity of the muscle to mechanical stimulation, biopsy material, unless it is allowed to lose its contractility before fixation, often shows fragmentation and contraction bands and thus the possibility of artifacts must be considered. A number of

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FIG. 7. Motor neurone disease. Typical neural atrophy. Note that the atrophic muscle fibers are in large groups and that the intact fibers in center appear almost normal. (Transverse section, sternomastoid. Iron hematoxylin and van Gieson. Magnification × 80.)

FIG. 8. Dystrophia myotonica in man aged 34, with family history. The muscle fibers are rounded, with central nuclei, and there is unusually severe fibrosis. (Transverse section, peroneus longus, iron hematoxylin and van Gieson. Magnification: × 350.)

FIG. 9. Dystrophia myotonica in man, aged 66, with family history. Note uneven size and rounding of muscle fibers, with central nuclei but no fibrosis. (Transverse section, tongue. Hematoxylin and eosin. Magnification × 80.)

FIG. 10. Dystrophia myotonica in man aged 66. Single muscle fiber showing multiple chains of muscle nuclei. (Longitudinal section, tongue. Hematoxylin and eosin. Magnification × 350.)

FIG. 11. Dystrophia myotonica in woman aged 48. There is a very long chain of central nuclei. (Longitudinal section, biceps brachii, hematoxylin and eosin. Magnification × 350.)

FIG. 12. Polymyositis in man aged 61, dying 6 weeks from onset of disease. Shows general disorganization of the muscle with necrosis of many fibers and interstitial cellular infiltration. (Longitudinal section, pectoralis minor. Hematoxylin and eosin. Magnification × 65.)

FIG. 13. Polymyositis, same case as Fig. 12. Severe acute degeneration. Segmental swelling and fragmentation of muscle fiber. (Longitudinal section, pectoralis minor, hematoxylin and eosin. Magnification × 350.)

autopsies have been performed on patients with dystrophia myotonica by Adie and Greenfield, 1923 (who give a very good and full account of the pathological changes in the muscles) Rouquès, 1931 Bielschowsky *et al.*, 1933 Black and Ravin 1947 Wohlfart, 1951 and others. Dystrophic changes in the myocardium have not been reported. The endocrine organs have been studied by some authors (Black and Ravin 1947 Thomasen, 1948)

D Myotonia Congenita

This rare congenital and hereditary disease was first described by Thomsen (1876) who himself suffered from it. It is in most cases nonprogressive and the muscles remain strong creatine and creatinine excretion are normal. Myotonia is widespread and a myotonic electromyogram is obtained from many muscles. Some authors (Maas and Paterson, 1939 1950) consider that this disease is a variant of dystrophia myotonica. Paramyotonia (Eulenburg 1886) in which the myotonia is only observable after chilling appears to be only a variant of Thomsen s disease (Thomasen 1948)

Little work has been done on the pathology of Thomsen s disease. The myotonic muscles are enlarged and strong the lower limbs characteristically showing most change, but the whole musculature may be hypertrophied Histologically the chief abnormal finding (in biopsy material) has been enlarged muscle fibers, with a diameter often well over 100μ. There may also be some tendency to rounding in cross section and a few central nuclei (Wohlfart, 1951 Greenfield *et al.*, 1957) Post mortems have been reported by Erb (1886) and Dejerine and Sottas (1895)

E. Other Muscular Dystrophies

1 *Ocular Dystrophy*

This condition has been described by Kiloh and Nevin (1951) and the literature fully reviewed. The disease appears to be confined, at least in the early stages, to the extrinsic ocular muscles and perhaps the orbicularis oculi. There is said to be great variation in the size of the muscle fibers with some hypertrophied fibers and a variable amount of fat and connective tissue between the muscle fibers the nerves are

Synonyms Thomsen s disease, myotonia hereditaria.
Synonym Progressive dystrophy of the external ocular muscles.

normal The syndrome may be a variant of the Landouzy Dejerine type of muscular dystrophy

However interpretation of biopsies from eye muscles requires care for normally the muscle fibers vary greatly in size, are rounded and often have central nuclei (Cooper and Daniel 1949 Cooper *et al* 1955)

2. *Dystrophy of Late Onset*

A number of cases of muscular dystrophy of late onset which are difficult to classify have been reported. Nevin (1936) and Welander (1951) have reviewed the pathology of these cases.

Welander (1951) studied 249 cases of a condition which she called distal late hereditary myopathy The age of onset varied from 20 to 77 years. Muscle biopsies were obtained from 26 patients and autopsies were performed on 3 who had shown symptoms for from 9 to 16 years. The histological picture seen was typical of a primary myopathy with, in early cases, rounding and variation in size of muscle fibers and increase of sarcolemmal nuclei with some central nuclei. Later there was great increase in the interstitial fibrous and fatty tissue

Barnes (1932) was able to find details of 283 descendants of a man born in about 1749 who died in 1836 and who suffered from a form of myopathy in the latter part of his life The disease in this family was always of late onset. As young adults, those later to be affected were unusually strong and tended to excel at games. Many achieved a ripe old age The muscles first affected were usually the proximal limb muscles, in contrast with Welander's (1951) cases in which the distal limb muscles were most commonly affected Unhappily muscle for histological examination was obtained from only one member of this remarkable family The illustrations show the changes characteristic of any muscular dystrophy rounded swollen muscle fibers, excess of nuclei and in one field excess of interstitial fat and fibrous tissue. No evidence was seen of phagocytosis or lymphocytic infiltration.

Although the number of cases of late myopathy reported is comparatively small a considerable number of muscle biopsies reach the pathologist, from cases presenting as muscle diseases arising in middle aged and elderly patients Some of these are neural degenerations which are clinically atypical Of those which are true myopathies neither the clinical nor pathological classification is yet certain nor do the two coincide Clinically after separation of those subsequently

found to have carcinoma, there are a group of distal myopathies and a group with a proximal syndrome. Either of these may or may not have a family history of muscle disease, and may run a longer or shorter course. Pathologically some cases are histologically similar to a "myositis," while a few are both clinically and pathologically similar to the dystrophies of young people.

V MYOPATHIES OF INFANTS

A. Amyotonia Congenita

This disease has more names than pathology It is here used to signify a congenital or infantile disease of muscles, as opposed to Werdnig Hoffman s disease which is an infantile spinal muscular atrophy in which the muscle changes are secondary to those in the motor nerves. The two conditions have been much confused but there now appears no doubt that there is both a spinal muscular atrophy (Werdnig-Hoffman s disease) and an infantile myopathy (for discussion of this, see the clinical section of this book) Werdnig Hoffman s disease is progressive and fatal, whereas, as the name benign congenital myopathy suggests, in true amyotonia congenita there is a tendency to recover There are therefore extremely few necropsy reports on cases of myopathy i.e. true amyotonia congenita. Spiller (1905) Councilman and Dunn (1911) Lereboullet and Baudouin (1909) and Menges (1931) have reported such cases, as has also Turner (1949) Turner reported the post mortem findings in a case which was unusual in showing muscular wasting Histological examination revealed only slight changes in the less affected muscles, but in the more severely atrophied ones, there were scattered foci of necrosis and invasion of nuclei, while some fibers otherwise normal had chains of central nuclei. Dr Greenfield reported these changes as those of a chronic myopathy

Biopsies have usually shown no abnormality according to Walton (1957) although others have found small muscle fibers in biopsies from "flabby babies," but some at least of these are no doubt cases of Werdnig Hoffman s disease. At present, there is no diagnostic pathology for this condition.

Coërs and Pelc (1954) and Woolf and Till (1955) using intravital

Synonyms Oppenheim disease myatonia congenita benign congenital myopathy

staining of nerve fibers with methylene blue in muscle biopsies, have suggested that the motor end plates may be immature for the age of the infant, which is interesting as others have suggested that the lesion is primarily a delay in development of the muscle.

B. Congenital Nonprogressive Myopathy

A form of amyotonia congenita has been described by Shy and Magee (1956) as a new congenital nonprogressive myopathy. In this familial disease the proximal muscles of the limbs were most severely involved and wasting was not prominent. The pathological changes in the muscles are most striking and appear to be diagnostic. The muscle fibers have a central core of fibrils, running almost the whole length. Fibrils in this central core have a striking difference in staining reaction from the peripheral fibrils: with Gomori trichrome, the peripheral fibrils stain red, while the central core stains blue. The core also gives a more strongly positive periodic acid-Schiff reaction than the peripheral fibrils. Some very large muscle fibers, up to 240μ in diameter, were found, and these had a special tendency to show central nuclei in chains. There was a moderate increase in interstitial fat, but no increase in collagen. The nerves appeared normal.

C. Arthrogryposis Multiplex Congenita

As its name suggests, this disease consists of congenital deformities of the limbs with rigidity of the joints. It appears that this state may be due to at least two pathological conditions, one a disease of the nervous system, the other a primary disorder of the muscles. Adams *et al.* (1953) in humans, and Whittem (1957) in calves, found evidence of neural disease, and a myopathic change was seen by Gilmour (1946). More recently, Banker *et al.* (1957) found severe myopathic changes in two infants, so that the disease may clearly also be due to a congenital myopathy.

VI. POLYMYOSITIS AND RELATED CONDITIONS

Polymyositis is the name now generally accepted for another idiopathic disease of muscle (see the clinical section of this book, Chapter XI, Vol. 3, by Henson), and it does not here refer to the

Synonyms: Amyoplasia congenita, multiple congenital articular rigidities, myodystrophica congenita deformans, myodystrophia foetalis deformans, congenital contractures of the extremities.

myositis caused by bacteria, viruses, or parasites. There is general agreement that dermatomyositis is a variant of this disease with a skin eruption. The skin lesion may indeed overshadow the muscle disease, but primary degeneration of skeletal muscle appears to be a basic part of the pathology and the condition is therefore a true myopathy whose etiology is quite unknown. In this account, we have confined the use of the term polymyositis to the pure muscle disease and to dermatomyositis. The menopausal dystrophy of Shy and McEachern (1951) and some of the myopathies of late onset appear to fall within this group and have the characteristic pathology It must, however be emphasized that the name is misleading as the disease is not primarily inflammatory Acute cases, running a fatal course of a few months, or even weeks, may have myoglobinuria, but myoglobinuria may occur in any very severe muscle degeneration and of itself does not alter the diagnosis. A number of cases have been associated with malignant disease This and other pathological and clinical features have been

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FIG. 14. Acute polymyositis showing disintegration of muscle fibers. In the center is a muscle fiber undergoing active phagocytosis. (Transverse section, pectoralis major hematoxylin and eosin. Magnification × 350.)

FIG. 15 Acute polymyositis showing atrophic muscle fibers. The central dark fiber with a chain of nuclei, may be regenerating (Longitudinal section, pectoralis major hematoxylin and eosin. Magnification × 350.)

FIG. 16. Acute polymyositis. Segmental swelling and fragmentation. Muscle fiber on the left shows granular degeneration, that on the right has vesicular nuclei. (Longitudinal section, pectoralis minor iron hematoxylin and van Gieson. Magnification × 350.)

FIG. 17 Acute polymyositis. Disintegrating muscle fiber (above) complete loss of fibers (below) (Longitudinal section, pectoralis minor iron hematoxylin and van Gieson. Magnification × 350.)

FIG 18 Acute polymyositis showing floccular degeneration in two muscle fibers. (Longitudinal section, pectoralis major hematoxylin and eosin. Magnification × 350.)

FIG. 19 Acute polymyositis showing mixed cellular infiltration, including polymorphonuclear leucocytes and a thin, basophilic, regenerating muscle fiber (dark) (Longitudinal section, pectoralis major hematoxylin and eosin. Magnification × 350.)

FIG. 20. Chronic polymyositis in man aged 38. Muscle fiber showing hyaline degeneration (above) and floccular degeneration and phagocytosis (below). (Longitudinal section, hematoxylin and eosin. Magnification × 350)

FIG 21 Facio-scapulo-humeral muscular dystrophy same case as Figs. 3 and 4 Active phagocytosis in fiber of muscle which had not shown recent clinical deterioration. (Longitudinal section vastus medialis, hematoxylin and eosin. Magnification × 350.)

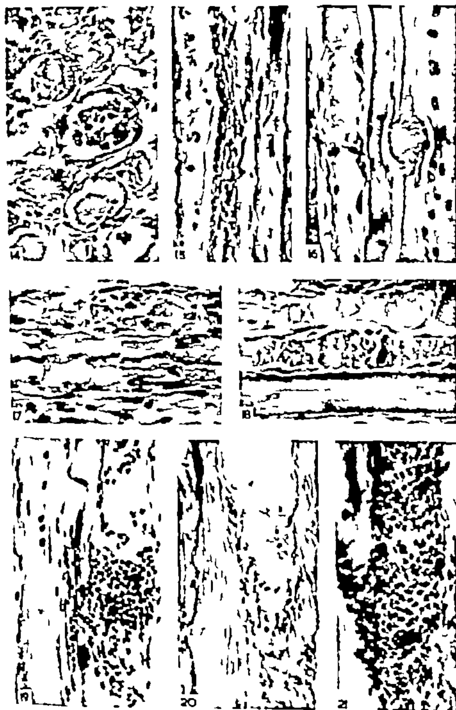


PLATE III

reviewed by Dowling (1955) and are fully treated by Walton and Adams (1958) in their monograph on the subject.

Affected muscles are macroscopically pale and they may be a striking gray white in color or have circumscribed areas of pallor which are often slightly swollen, or again pale flecks are sometimes present throughout the muscle in the most acute cases there may be small hemorrhages. Any muscle can be affected, but the trunk, neck, and proximal limb muscles are usually most severely involved the respiratory muscles, tongue, and muscles of deglutition also sometimes have lesions

Histologically the muscles show an acute myopathy The most striking changes are a degeneration of the muscle fibers, which are rounded vary markedly in size, show genuine changes in staining reaction, and have greatly increased numbers of nuclei (*Figs. 12, 13* and 14) Fibers may be necrotic throughout their length, but segmental degeneration is very typical, short lengths of fiber being swollen and without striations (hyaline or cloudy degeneration) (Fig 20) Fragmentation, often with areas of empty sarcolemmal sheath stretched between the fragmented ends of sarcoplasm is seen (Fig 13) Floccular degeneration (Fig 18) is common, and segments in which the sarcolemmal sheath is filled with nuclei (Fig 14) may be numerous. The finding of these last two changes is in fact almost a necessity for making a diagnosis. In the interstitial tissue lie both free sarcolemmal nuclei and many types of cell (Figs 12 19) — macrophages, plasma cells, lymphocytes, and a variable number of polymorphs including eosinophils The infiltrated cells may be present diffusely throughout the tissue or in collections between the muscle fibers and around vessels. Large collections of lymphocytes may be found, and curiously enough these are more often seen in the less degenerated areas of muscle. The muscle fibers are of all sizes some are obviously swollen (up to 200μ in diameter) but many are shrunken and some having lost all their cytoplasm, are represented only by chains of nuclei Mixed with the degenerating fibers are thin regenerating fibers (Fig 19) basophilic and containing vesicular nuclei, and sarcoplasmal giant cells (muscle buds) which are also probably regenerating fibers. Good illustrations of these various changes will be found in Kinney and Maher (1940) and Walton and Adams (1958)

Muscle spindles may be involved in the general degeneration. Blood vessels and nerves are normal (reports of neuromyositis have not been

substantiated) Infiltration with fat is not a feature There may be some fibrosis, but we have not found it to be very marked even in chronic cases.

This over all picture is very characteristic when present. Unfortunately the histology is extremely variable. Not all the muscles are affected in any one case and even a single muscle may have areas which are normal and areas of the severest damage. There may be little evidence of interstitial reaction or of regeneration and the muscle fibers may show only hyaline change and a mild increase in nuclei. Again, there may be only collections of round cells between normal fibers. Neither of these last two appearances are diagnostic and the interstitial nodular myositis" is more characteristic of other conditions such as rheumatoid arthritis and myasthenia gravis.

The heart may show small foci of myocardial degeneration with lymphocytic infiltration, or a patchy fibrosis. The kidney tubules, in those cases with myoglobinuria, may contain myoglobin. In dermato-myositis, the skin shows atrophy of the epidermis with thickening and condensation of the underlying collagen, sometimes with vascular proliferation and focal infiltration with cells.

The more chronic cases of polymyositis cannot always be distinguished from muscular dystrophy (in which incidentally skin changes have many times been reported) In our experience increase in collagen has not been very great in polymyositis and severe fibrosis would favor a diagnosis of dystrophy Muscle fibers with segments of acute necrosis and interstitial infiltration may be found in muscular dystrophy (Fig 21) but in dystrophy there is usually less acute degeneration and no regeneration.

Some authors do not distinguish between polymyositis and scleroderma, considering them both to be varieties of the group of conditions which have been called "collagen diseases." In scleroderma however visceral lesions are the rule (Dowling 1955) particularly atrophy of the plain muscle of the gastrointestinal tract and arterial lesions especially affecting the kidneys. This is not so in typical polymyositis A review of the literature on the pathological changes in scleroderma will be found in Dowling (1955) who also considers that the skin lesions in the two conditions are different. The muscle changes in scleroderma may no doubt sometimes be identical with those in polymyositis, but in our admittedly limited experience the muscle lesions in scleroderma have been more purely degenerative than in polymyositis, with little

cellular reaction and no muscle buds present. Furthermore, the histological appearances of polymyositis may be produced experimentally in a number of unrelated ways, e.g by poisons, and are probably simply the picture of any acute muscle degeneration. Therefore it would seem to us best to classify the conditions separately for the present.

Polymyositis, at least, appears to be a primary muscle fiber degeneration and not a degeneration secondary to disease of the interstitial collagen so that there seems little reason to call it a "collagen disease.

VII. MUSCLE DISEASES ASSOCIATED WITH SYSTEMIC AND METABOLIC DISORDERS

A. Myopathy Associated with Malignant Disease

Patients suffering from carcinoma frequently develop muscular weakness and when the clinical signs are severe enough to suggest muscle disease, these cases form, clinically part of the heterogeneous group of "late myopathies." Denny Brown (1948) Henson *et al.* (1954) and Heathfield and Williams (1954) have reported somewhat indefinite pathological changes There is also an undoubted association of dermatomyositis and carcinoma (Domzalski and Morgan, 1955 Walton and Adams, 1958) The changes found in malignant disease usually consist of simple atrophy of muscle fibers and an increase in nuclei. Sometimes a more acute degeneration of muscle fibers with some cellular infiltration is seen, resembling a mild polymyositis.

Carcinomatous neuropathy is well recognized and we believe that the muscle lesions seen may be partly accounted for by a scattered neural degeneration.

Little is known of the changes produced in human muscle by prolonged bed rest, cachexia, and senility but that considerable changes occur in the muscles of such cases is certain Until we know more of these matters, the significance of the changes seen in the muscles of patients with carcinoma must remain in doubt.

B Periodic Paralysis

The attacks of paralysis, from which the disease is named, are accompanied in most cases by a fall in serum potassium, associated with a decrease in urinary excretion of potassium (i.e a retention of potassium in the body as a whole) The relationship to potassium metabolism is not, however a simple one, as serum potassium may not be lowered during attacks (Tyler *et al.*, 1951) and may even be raised

(Bull *et al.* 1953) Conn *et al.* (1957) have shown that sodium retention may be the precipitating factor. Whether periodic paralysis should be considered as one disease or several, the pathology is the same in all and vacuoles are formed within the muscle fibers (Goldflam, 1897, Tyler *et al.*, 1951, Conn *et al.* 1957). The fibers may be distended by droplets which resemble in lesser degree those seen in von Gierke's disease, they do not, however, contain glycogen.

C. Glycogen Storage Disease

A condition which may cause difficulty in diagnosis because of its remarkable clinical similarity to amyotonia congenita is a form of glycogen storage disease in which there is profound muscular weakness due to infiltration of the skeletal muscle fibers with glycogen. The diagnosis can be made readily by muscle biopsy but without the aid of this device, it may be impossible to differentiate between the two diseases. Humphreys and Kato (1934) illustrated the pathological changes of the striated muscle fibers in this form of von Gierke's disease. The muscles involved show marked pallor and histologically the muscle fibers show a curious form of vacuolation. In an advanced case, the vacuoles, which are filled with glycogen, replace all the sarcoplasm in the fiber so that only a swollen sarcolemmal sheath is seen. In the lesser degrees of involvement there may merely be small vacuoles, filled with glycogen, in the middle of the muscle fibers. The remaining nuclei in badly affected fibers may be pyknotic. Gunther (1939) found that a case diagnosed clinically as one of amyotonia congenita, had deposition of glycogen in vacuoles in the skeletal muscles. Di Sant Agnese *et al.* (1950) found that when glycogen storage disease affects the heart, the skeletal muscles are also often affected. Krivit *et al.* (1953) in an interesting paper on three infants (siblings) all with a flaccid weakness of the skeletal muscles, describe the post mortem findings on two of the children and illustrate the pathological changes in the muscle fibers. Clinically, the three children resembled cases of amyotonia congenita, and these authors stress the necessity for muscle biopsy in the diagnosis of the muscular atrophies of infancy.

D. Polyarteritis Nodosa

Polyarteritis nodosa is not primarily a muscle disease but is included here since the muscles are probably more commonly involved than

Synonym: Periarteritis nodosa.

any other tissue and muscle biopsies from suspected cases are often presented for diagnosis. The diagnostic finding is arteritis of an intra muscular arteriole. The vessel wall and perivascular space are infiltrated by acute and chronic inflammatory cells. Fibrinoid necrosis may be demonstrable and there may be thrombosis. The effects upon the muscle are essentially due to ischemia which causes either a direct degeneration of muscle fibers or a secondary degeneration owing to the involvement of nerves. Multiple small infarcts are found, with hyaline eosinophilic muscle fibers and also groups of small atrophic muscle fibers resulting from neural atrophy. Later the dead fibers are removed and a patchy fibrosis of the muscle is seen. Perivascular or interstitial collections of cells may be the only finding but this alone is not diagnostic.

E. Thyroid Disease

1. *Hyperthyroidism*

Muscular weakness, wasting and fascicular twitchings occur commonly in thyrotoxicosis and this muscular weakness may precede other symptoms though it is not necessarily proportional to the basal metabolic rate. If the muscular symptoms are pronounced enough, they may constitute a myopathy (chronic thyrotoxic myopathy). The pathology has been little studied but as long ago as 1898, Askanazy reported an infiltration of fat between the muscle fibers and atrophy of the fibers themselves. Adams *et al.* (1953) found the same changes and we have seen a muscle biopsy which showed a histological picture very similar to their illustrations. The marked increase in fat is interesting as subcutaneous fat is reduced in thyrotoxicosis. The condition is known as chronic thyrotoxic myopathy to distinguish it from acute thyrotoxic myopathy a very rare clinical syndrome of which the pathology is not known.

2. *Hypothyroidism*

Enlarged muscles with slow movements may be found in hypothyroidism both in adults and children. In myxedematous adults, the condition is called Hoffman's Syndrome (Thomasen, 1948) and in cretins, Debré and Semelaigne's Syndrome (1935). In cretins, enlargement of the tongue is a usual feature. In spite of the clinical enlargement of the muscles, little is known of the pathology hypertrophy of the muscle fibers has not been adequately substantiated.

F Exophthalmic Ophthalmoplegia*

The pathology of this condition in which exophthalmos is accompanied by paralysis of the external ocular muscles, has been studied by Burch (1929) Naffziger (1933) Brain and Turnbull (1938) and Mulvany (1944) The pathological features are macroscopic enlargement sometimes very marked, of the external ocular muscles which are pale and firm, while microscopically the muscle fibers show a non specific atrophy in which focal degenerative changes are mild En larged fibers may be present (Brain and Turnbull, 1938) The main changes are in the interstitial tissue and consist of edema severe fibrosis in the later stages, and a variable degree of lymphocytic in filtration. These cellular infiltrations may be massive (Burch 1929)

The macroscopic enlargement of the muscles appears to be due to edema as it is not accounted for by hypertrophy of the fibers. The disease is related to increased activity of the thyroid but thyrotoxicosis is not a necessary accompaniment. It is thought to be due to over activity of the pituitary producing an excess of thyrotrophic hormone

VIII. MYASTHENIA GRAVIS

It was believed for a considerable period of time that the sole histological changes to be found in the striated muscles of cases with myasthenia gravis were the so-called "lymphorrhages." That this is not the case was shown clearly by Russell (1953) though much earlier Buzzard (1905 1910) had noted that in addition to "lymphorrhages," there were slight degenerative changes in the muscle fibers in this disease, though well marked atrophy of muscles was rare.

Russell (1953) described a necrosis of muscle fibers, with swelling of the fibers and eosinophilia of the sarcoplasm followed by the development of an inflammatory exudate in and around the fiber In examples of muscle lesions where lymphorrhages were most prominent a progressive atrophy of muscle fibers was seen the nuclei often be coming central In other specimens there was simple atrophy of single muscle fibers and groups of fibers Walton and Adams (1958) think that there is a similarity between the pathological changes described by Russell (1953) and those found in cases of polymyositis. Rowland *et al* (1956) review some 26 post mortem reports on cases of myasthenia

* Synonyms Progressive exophthalmos malignant exophthalmos thyrotrophic exophthalmos.

gravis and Woolf *et al.* (1956) describe changes in the intramuscular nerve endings in one case.

It would seem that most of the pathological changes seen in the muscle fibers in cases of myasthenia gravis are the nonspecific reaction of these fibers to some injury or metabolic disturbance at present unknown.

IX. MYOGLOBINURIA

Myoglobinuria occurs either as a spontaneous, paroxysmal disease or "symptomatically" as a result of the rapid destruction of a large quantity of muscle, from any cause. Björck (1949) describes the various conditions in which myoglobinuria is found. In the "crush syndrome," myoglobinuria follows extensive trauma to muscle and leads to anuria (Bywaters, 1944) and a similar condition may result from extensive infarction of muscle (Bywaters and Stead, 1945). It is well known that myoglobinuria may be seen in severe dermatomyositis and that it occurs occasionally in cases of muscular dystrophy (Acheson and McAlpine, 1953). Idiopathic paroxysmal myoglobinuria is a rare condition in man; it is sometimes familial. In Germany outbreaks of paroxysmal myoglobinuria (Haff disease), thought to be due to eating poisoned fish, have occurred and the condition has been reviewed by Günther (1940). Paroxysmal myoglobinuria is a well known condition in horses, developing after good feeding and unusual exertion; march "hemoglobinuria" in man may possibly be a related condition. The pathology of idiopathic paroxysmal myoglobinuria has been little studied but Schaar *et al.* (1949) performed a necropsy on a case and Elek and Andersen (1953) reported a muscle biopsy and discussed the literature. The pathological changes appear to consist of acute degeneration and necrosis of the muscle fibers, followed by regenerative changes, so that it much resembles polymyositis.

X. MYOPATHY IN ANIMALS

Degeneration of the skeletal muscles in animals is not rare. The common muscular degenerations, however, are acquired conditions related to dietetic and environmental factors. In the veterinary literature these are described as "muscular dystrophies," though they bear no resemblance to the conditions known as muscular dystrophy in the human. The animal myopathies which do resemble muscular dystrophies in the human are exceedingly rare.

Michelson *et al.* (1955) have described, as dystrophia muscularis, a hereditary primary myopathy in the house mouse, which has many of the features of a human muscular dystrophy The disease develops early in life, beginning in the hindlimbs and running a slowly progressive course to involve the trunk and forelimbs. At necropsy the nervous system has been found to be normal while the histological appearance of the muscles is strikingly similar to that seen in a human muscular dystrophy There is rounding and atrophy of muscle fibers, which have some central nuclei, no evidence of regeneration, and an increase of fibrous interstitial tissue. The illustrations in this paper might well be taken from a human case.

One example of a muscular dystrophy in animals has been extensively studied. This is the congenital myotonia of goats which was fully described by Kolb in 1938 he considered that the condition was essentially the same as myotonia congenita (Thomsen's disease) Unfortunately the pathological changes in these animals were not fully studied, but the illustrations show rounding of muscle fibers and central nuclei, suggestive of a dystrophy A number of physiological studies have been made on these myotonic goats by Brown and Harvey (1939) who concluded that the myotonia was in every way similar to myotonia in man and that the primary dysfunction was in the muscle fiber

Innes (1951) in a paper in which he reviews what little is known of myopathies in animals, describes a case of possible muscular dystrophy affecting the gastrocnemii of a dog in which the histological changes were typical of a true muscular dystrophy in man. Ziegler (1929) described a somewhat similar condition in a dog with generalized disease. Bosanquet *et al.* (1956) have described a myopathy occurring sporadically in adult sheep under good nutritional conditions. The condition appears to be fairly common, as they found 45 cases. Affected muscles were distributed all over the body and histologically the changes resembled a polymyositis, though the chronicity of the condition and the possibility of an inherited factor are thought to be more suggestive of a dystrophy

It was pointed out earlier that one of several etiological factors may induce the condition of arthrogryposis multiplex congenita in the human. This is probably true in animals also, for Whittem (1957) has suggested that in calves it has a neurogenic etiology while one form of the condition in sheep is thought to be myogenic (Middleton, 1934)

Paralytic myoglobinuria of horses is a well-known condition. It is a paroxysmal disease which follows severe exertion after rest. At necropsy, the muscles are salmon pink in color and histologically show acute hyaline degeneration and necrosis of muscle fibers (Carlström, 1931, Minett, 1935). Myoglobinuria is also known to occur in various animals suffering from severe "dystrophy," where it is apparently secondary to the muscle degeneration and comparable with "symptomatic" myoglobinuria in human polymyositis. Myoglobinuria which follows transportation or driving may however possibly be related to the paroxysmal disease of horses. The pathology in all these conditions is that of a nonspecific acute degeneration of muscle.

A number of animal diseases are described in the literature as "muscular dystrophies" but these do not resemble, either pathologically or clinically, the human muscular dystrophies, being pathologically more like human polymyositis, and Ahlström (1948) in fact uses this term. Frauchiger and Fankhauser (1957) go so far as to say that, in their opinion, in no species is there any disease comparable with the human myopathies and dystrophies. Many descriptions of animal myopathies suffer from having no adequate pathological examination. The so-called "muscular dystrophies" of animals have been reviewed by Blaxter and McGill (1955) and Hartley and Dodd (1957) who point out that many have a nutritional basis. The nutritional aspect of muscle disease is covered by Mason in this volume.

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CHAPTER XI

Clinical Aspects of some Diseases of Muscle

RONALD A. HENSON

I. INTRODUCTION

Although clinical interest has been mainly centered on those forms of muscle disease which are either hereditary or associated with disorders of excitation and contraction it is worth recalling that the common affections of muscle are far less mysterious. For example, from a global point of view there can be no doubt that infection, trauma, and circulatory disturbances are responsible for a high proportion of muscular disorders. Acute and chronic pyogenic inflammations are common in the tropics, though rare in more temperate zones. Infestations with parasites are also mainly tropical or subtropical in their distribution though such conditions are by no means

beyond the experience of those who practice in cooler climes. Traumatic lesions, due in the main to direct physical injury, are probably even more numerous. Ischemic conditions, generally the fruit of degenerative vascular disease, form a major source of disability. Cachexia due to new growth or other serious systemic disease is associated with generalized muscular atrophy, while localized wasting and weakness occur in muscles around injured or diseased joints. It is a matter of almost universal experience that inactivity, frank disuse, and increasing age lead to muscular enfeeblement and atrophy. Lastly, any condition interfering with the functions of the lower motor neuron may produce muscular paralysis and wasting. These simple facts have been recited to bring into proportion those more esoteric conditions, such as muscular dystrophy, periodic paralysis, and the myasthenias, which generally spring to mind when the subject of diseases of muscle arises.

TABLE I

CLASSIFICATION OF DISEASES OF MUSCLES

Congenital defects
Muscular dystrophies
Inflammatory diseases
  A. Specific
    Bacterial, viral, granulomatous, fungal, parasitic
  B. Non-specific
    Toxic myopathy (Focal muscle necroses in acute and chronic infections)
Vascular diseases
  e.g. Arteriosclerosis
Collagen-vascular diseases
  Disseminated lupus erythematosus, scleroderma, rheumatoid arthritis, etc.
  ?dermatomyositis and polymyositis
Diseases due to physical agencies
  Traumatic conditions, effects of cold
Myopathies associated with Endocrine Disorder
  e.g. Thyrotoxic myopathy
Myopathy associated with Carcinoma
Metabolic disorders
  Glycogen storage disease
Miscellaneous atrophies
  Disuse, senility, nutritional deficiency, atrophy resulting from malignant and other serious systemic diseases
Disorders of excitability and contractility
  The myasthenias, periodic paralysis, other disorders of potassium metabolism
Tumors
Neural atrophies

Table I represents an admittedly incomplete attempt to classify diseases of muscle The list is not intended to be comprehensive. It has been compiled on an etiological basis where the causes are known otherwise a clinicopathological scheme has been employed. The

TABLE II

ANALYSIS OF CASES OF MUSCLE DISEASE, NEUROLOGICAL DEPARTMENT THE LONDON HOSPITAL, 1948–1957

Disease	Number of cases
Muscular dystrophies	
Duchenne variety	8
Facio-scapulo-humeral variety	14
Other limb girdle dystrophies	16
Ocular	5
Localized	3
Distal type	1
Muscular dystrophy with myotonia	
Dystrophia myotonica	16
Myotonia congenita	1
Total muscular dystrophies	64
Amyotonia congenita syndrome	5
Infections	
Epidemic myalgia	2
Collagen-vascular diseases	
Dermatomyositis and polymyositis	17
Scleroderma	2
Traumatic and vascular conditions	
Anterior tibial syndrome	2
Myopathies associated with thyroid disease	
Exophthalmic ophthalmoplegia	33
Thyrotoxic myopathy	8
(Myasthenia gravis) (3)	
Myopathy associated with carcinoma	14
Disorders of excitability and contractility	
Myasthenia gravis	42
Periodic paralysis (with and without low serum K)	5
Other disorders of K metabolism	2
Others	
(1 proximal muscular syndrome cause undetermined, 1 neuromyositis)	2
Total patients	200

Cases of vascular disease, trauma, neural atrophy and miscellaneous atrophies excluded.

approach is essentially a practical one. For example, a group of cellaneous atrophies have been arbitrarily placed together The muscular dystrophy has been reserved for patients with progre muscular disease of genetic origin. Myopathy is used in its prir sense, disease of muscle, only Table II shows the distributior diagnosis of cases of muscle diseases seen in the Neurological De ment of The London Hospital and in personal practice, during past 9 years. This Table is given simply as an indication of the pers experience on which this chapter is based It reflects the interests o physicians to the department, and this is seen particularly in rather large number of cases of exophthalmic ophthalmoplegia. Dis of muscle accounted for less than 1% of patients seen during the years.

There is obviously no need for discussion of the more com muscular affections which have been mentioned Many of them well understood and treatment is effective. Nor shall we con muscle dysfunction resulting from neurological disorders, save few contexts. The conditions described form a rather heterogen group which are either of particular clinical or theoretical interes in which recent advances in knowledge have occurred. In mo these complaints, the muscular system is diffusely affected, sometim a result of a primary myopathy sometimes as a manifestation more generalized disease process.

II. SOME GENERAL CLINICAL FEATURES

Muscular development varies among healthy individuals accor to age sex, race, and other factors. The general state of health nutrition are reflected in the condition of the muscles. Further mu soon waste and become weak if they are not used. Some clinical perience is therefore necessary to enable the physician to de whether muscular atrophy is due to neuromuscular disease or to a more general or banal cause For example, the evaluation of musc wasting and weakness in the elderly or infirm may be difficult

The symptoms of the forms of muscle disease under consideration largely variations on a theme of muscular weakness. In the musc dystrophies, and certain other important myopathies, the weakne commonly predominant in the limb girdles and trunk, the so-ca proximal muscular syndrome Muscular atrophy due to disease of

lower motor neuron, on the other hand generally begins in the distal muscle groups, as for example in motor neuron disease and peripheral neuropathy There are exceptions in both types of case. Poliomyelitis may of course, produce any pattern of neural muscular atrophy Certainly subacute or chronic proximal muscular atrophy leads to the initial suspicion that the condition is myopathic.

The relationship between the degree of atrophy and weakness varies in different muscular affections. Where there is widespread wasting in an ambulant patient with malignant disease or some chronic infective process, without a specific neural lesion, general reduction in power is found but in the earlier stages, at least, power is often surprisingly good, considering the degree of atrophy The relationship between muscle bulk and power is complicated in the muscular dystrophies by the occurrence of pseudohypertrophy This unfortunate term denotes muscle enlargement without increased power or more popularly with actual weakness. Pseudohypertrophy is seen in its extreme form in a child dying from the Duchenne type of muscular dystrophy To the eye, the contours of the muscles are well maintained, or even exaggerated, but they are unusually soft to palpation. Post mortem examination shows the affected muscles to be largely or even entirely replaced by fat and connective tissue. In the earlier stages of the disease, however the enlarged muscles are firm to the touch and often less weakened than those which are visibly atrophic. In some forms of muscular dystrophy enlarged muscles have increased power clinically a true hypertrophy However muscle biopsy in such a case may reveal severe degenerative changes, and certainly these apparently hyper trophic muscles usually grow weak with the passage of time. Until the pathology of pseudohypertrophy and hypertrophy is more fully understood it would be better to abandon the terms and speak of muscular enlargement, with normal, increased, or diminished power as the case may be.

This is a convenient point at which to define myotonia and myasthenia, two classical symptoms of some muscle diseases. Myotonia is a condition in which the affected muscles continue to contract after voluntary contraction has ceased It occurs in response to mechanically stimulated contraction, as by percussion or electrical stimulation Voluntary contraction itself may be slowed down. Myotonia is usually worse in states of cold or fatigue. It may be diminished by repeated movement of the affected muscles, though this is not always the case.

C. Clinical Features

1 *General*

The patient with muscular dystrophy presents with complaints stemming from muscle weakness, though the term weakness is rarely mentioned by the patients or parents. Thus, parents may say that a child has never been able to walk, that his gait is clumsy or that he tends to fall without cause. Specific defects may be mentioned, for example, difficulty in rising from a chair in mounting stairs, or in lifting the arms. Dragging of the legs may be noted. A spinal deformity may be the reason for consultation Not surprisingly children with muscular dystrophy are sometimes referred to an orthopedic surgeon. In adults, the range of complaints is wider but the underlying pattern is the same. The weakness usually results from affection of the limb girdles and trunk. The onset is insidious, and diagnosis is usually obvious by the time the patient comes for examination. Examination will show muscular wasting and weakness, commonly the proximal muscular syndrome, with or without muscle enlargement. The tendon reflexes are generally reduced in the affected parts. There are no signs of a nervous lesion. Skeletal deformities, for example increased lumbar lordosis or spinal kyphosis, may be present. A detailed review of the clinical aspects of the muscular dystrophies has been made by Walton and Nattrass (1954) and their paper contains an extensive bibliography The original accounts by early workers are referred to elsewhere in the text.

2 *Duchenne Type (Synonym Pseudohypertrophic muscular dystrophy)*

This form of muscular dystrophy was described by Duchenne (1868) but he did not recognize that the paralysis was myogenic. Although muscular enlargement (pseudohypertrophy) frequently occurs in this variety the term pseudohypertrophic muscular dystrophy is best avoided as a similar increase in muscle bulk occurs in other forms, and it is not invariably present in patients who otherwise appear to belong to this group (Stevenson, 1953 Walton and Nattrass, 1954) This is generally an affection of young children, occurring almost exclusively in males. It is inherited as a sex linked recessive. The onset may be remarked by parents in the first year of life, and before the age of four in one half of all cases (Walton and Nattrass, 1954) Occasionally the onset may be as late as the third decade. The muscular weakness and wasting begin symmetrically in muscles of the pelvic girdle and thighs,

especially the iliopsoas, quadriceps gluteus maximus, and sacrospinalis. The child walks with a waddling gait and has difficulty in climbing stairs or rising from a chair If asked to rise from the ground he "climbs up himself" in characteristic fashion. The anterior tibial group and peronei are involved later The calf muscles, hamstrings, and abductors of the thighs are relatively spared, and this accounts for the muscular contractures which are liable to develop After a time, the shoulder girdles and upper trunk muscles become affected and later still the arms and forearms. The calf muscles are commonly enlarged and firm Rarely this enlargement is shared by the quadriceps, hamstrings, glutei, deltoid, or other muscles in the shoulder girdle and upper limb As already noted, the enlarged calf muscles retain their power remarkably well in comparison with other muscle groups. Nattrass (1957) confirms this view The enlargement may disappear later or the muscles may become less firm to the touch.

This complaint is inexorably and, for a muscular dystrophy rather rapidly progressive. Contractures appear immobility leads to severe osteoporosis and gross skeletal deformities. Remissions or periods of arrest do not occur Death results from inanition or respiratory infection, usually in adolescence. A few patients survive into the third or fourth decade, in a state of helplessness. The course is similar in older patients.

3 *Facio-Scapulo-Humeral Type*

This variety was first described by Landouzy and Déjèrine (1884) It has been regarded by some as the most distinctive variety of muscular dystrophy Onset is commonly early in the second decade, but it may be earlier or later It is rarely possible to determine the precise length of the history in this insidious complaint. Males and females are affected. The muscles of the face are involved at an early stage The lips tend to be everted, forming the so-called tapir mouth. The patient cannot whistle. Attempts to smile produce a sneer due to weakness of the zygomatici. Later eye closure and wrinkling of the brow become enfeebled The presenting symptoms, however are referable to the atrophic palsy of the shoulder girdle, arms and trunk. Spinati, rhomboids, serratus anterior pectoralis major deltoid, biceps, and triceps are affected early the scapulo-humeral muscles in fact. The disturbance may be asymmetrical Trunk weakness is usually prominent, and this leads to an exaggerated lordotic stance. The complaint is slowly progressive, though there may be long periods of arrest. After a few

D Muscular Dystrophy and Myotonia

The clinical features of myotonia have been previously described. Myotonia is commonly associated with muscular dystrophy in the condition known as dystrophia myotonica, but it also occurs in myotonia congenita, a much rarer complaint, and in hereditary paramyotonia. The relationship between these three complaints has been widely discussed. Maas and Paterson (1939, 1950), writing from wide experience, hold the view that these three syndromes are all manifestations of the same disease process. Others hold an opposite opinion (e.g. Adams *et al.* 1953, Bell, 1947).

1 *Muscular Dystrophy with Myotonia* (*Synonyms: Dystrophia Myotonica, Myotonia Atrophica*)

In this hereditary disease, muscular dystrophy is linked with myotonia and other dystrophic disorders, such as cataract and gonadal atrophy. As a rule, cataract is the only abnormality present for several generations, and then in one generation, the whole dystrophic disturbance appears. The myotonic dystrophy may be repeated in following generations, appearing at an earlier age. This is the phenomenon of anticipation, which is also shown by the progressively earlier appearance of cataract in preceding generations.

Dystrophia myotonica occurs in both males and females. The onset is commonly between the ages of 15 and 40, but it may occur in childhood. Recognition of the condition may be delayed until old age, usually because the patient has avoided consultation, though symptoms have been present for many years. The characteristic features are the myotonia, which is usually limited to the hand, forearm, and tongue, and muscular atrophy and weakness. The wasting is commonly conspicuous in the facial muscles, sternomastoids, muscles of the shoulder girdles, forearms and hands, quadriceps, and the muscles of the legs below the knees. Ptosis is present in about a half of all cases. Emaciation, impotence, testicular atrophy, amenorrhea, baldness, excessive perspiration, and mental defect are other features. Affection of the pharyngeal muscles may lead to dysphagia. Dysarthria is a common feature being due to myotonia in the tongue muscles. The diagnosis can usually be made at sight from the characteristic expressionless facies, hollowed temples and cheeks, drooping eyelids, and baldness. Muscular enlargement is rare. Disturbances of atrioventric-

ular conduction are present in some patients (Evans, 1944) The condition is progressive, leading to severe disability, generally within 20 years. The patient usually succumbs in late middle age.

The myotonia is increased by neostigmine and diminished by quinine and procaine amide. Brown and Harvey's (1939) work suggest that the disorder is primarily muscular

2. *Myotonia Congenita (Synonym Thomsen's Disease)*

This condition was described by Thomsen (1876) who himself suffered from the disease. The myotonia is probably congenital and is usually observed in childhood Males and females are affected The myotonia, which may be generalized or localized, is associated with exceptional muscular development, and increased muscular power There is no muscular wasting and the reflexes are normal The basic symptom is an interference with movement through tonic muscular spasm. An attempt at a forceful movement from rest, or a change from a state of easy activity to strong muscular contraction produces painless cramp of the muscle group involved. The patient is truly "muscle bound" Emotional disturbance cold, or fatigue tend to bring on the myotonia. The patient is able to "loosen up" so to speak, by a series of laborious movements of the affected part. During the spasm the muscles are prominent to the eye and hard to the touch Life is not shortened. Some patients with myotonia congenita develop muscular wasting and weakness earlier or later in life, and this is one of the facts which has led some to suppose that the condition is simply another manifestation of the dystrophia myotonica syndrome The severity of the myotonia tends to decrease as the patient grows older

3 *Hereditary Paramyotonia*

In this form of myotonia, the symptom only occurs on exposure to cold

4 *Myotonia Acquisita*

This term has been used to describe cases of myotonia coming on in adult life, in the absence of a family history of the symptom.

E. The Differential Diagnosis of Muscular Dystrophy

"The diagnosis of muscular dystrophy rests on the onset, usually at an early age, of symmetrical muscular wasting with a distribution which cannot be explained in terms of the innervation of muscles"

(Brain, 1955) A family history of similar illness is helpful. The presence of "pseudohypertrophy" is pathognomonic. Difficulty may arise when the onset is in infancy and this problem is discussed in the section on the amyotonia congenita syndrome. In older patients, the diagnosis is usually easy by the time medical help is sought.

Differentation from motor neuron disease causes no difficulty the age of onset, presence of fasciculation reflex changes, and tempo of the paralysis form obvious points of difference. Rare cases of very chronic spinal motor neuron degeneration can usually be distinguished by the presence of fasciculation. Some patients with longstanding myasthenia gravis (q v) develop considerable muscular atrophy and the distinc tion here must be made on the basis of the response to the injection of neostigmine sulfate or edrophonium chloride Poliomyelitis, chronic forms of peripheral neuropathy, and peroneal atrophy are usually mentioned as requiring differentiation but the dissimilarities in each instance will be apparent.

Recently, emphasis has been placed on the need to distinguish polymyositis (q v) from muscular dystrophy Both are conditions of which the cause is unknown both commonly present with the proximal muscular syndrome. The natural histories, and particularly the tempi, of these illnesses are so different, as the clinical accounts show that confusion is unlikely to arise However the use of muscle biopsy in the investigation of muscular dystrophy has brought difficulties in its train. The histopathological criteria for the diagnosis of polymyositis include the demonstration of focal muscle necrosis, with leucocytic infiltration, and basophilic fibers with centrally placed vesicular nuclei and prominent nucleoli, which have been interpreted as points of regeneration. In our experience, these changes have been found in the muscles of patients who were clinically classic examples of familial muscular dystrophy and particularly in two members of one family with muscular enlargement ... increased power This is presumably a reflection of the fact that ... limits to ... in which muscular tissue can respond to ... than an indication of any ... the ... conditions. Incidentally the intensity ... related to the acuteness of ... myositis should be c... y rapid in their onset, parti...

children, the onset of a rapid, apparently dystrophic, process should be viewed with suspicion, especially if the victim is a girl. Other points will become apparent when the subject of polymyositis is considered

The diagnosis of the myotonic disorders is simple. Mistakes may conceivably be made in patients with dystrophia myotonica in whom the myotonic element is minimal.

F The Treatment of Muscular Dystrophy

Many forms of treatment have been tried, and these have been surveyed recently by Walton and Nattrass (1954) and Nattrass (1957) It is notoriously difficult to assess the effects of treatment in chronic complaints which may be characterized by periods of spontaneous arrest. It can be said that there is no treatment which may be relied upon to retard the progress of muscular dystrophy Treatment is entirely symptomatic and supportive.

Merritt (1952) reported unfavourably on the use of adrenocorticotropic hormone and cortisone. I have used cortisone in the treatment of five cases, four of facio-scapulo-humeral dystrophy aged 16 17 20, and 42, and one of a limb girdle type of late onset, aged 40 All were familial cases. Cortisone was used because muscle biopsy showed changes which could be interpreted as "inflammatory " It was felt at that time that these changes might indicate an active phase of the dystrophic process. The two older patients claim improvement and have been taking cortisone for some 3 years In neither case does formal examination reveal any clear increase in power of any muscle or muscle group but performance has improved. Thus a woman, aged 42 with facio-scapulo-humeral dystrophy was able to get upstairs for the first time in 3 years on returning home after starting treatment and a man aged 40 with a limb-girdle dystrophy was enabled to carry on his business as a pastrycook when he had been on the point of retiring because of his weakness. Both these patients protested volubly when attempts were made to withdraw the cortisone after a short course of treatment, and they remain unwilling to forego it. None of the three young patients, including the son of the woman who benefited, experienced any improvement whatsoever On these results, it has been decided not to use cortisone again, especially in view of the problem of addiction.

Although there is no treatment which can aid the weakness of dystrophia myotonica, the myotonia can be reduced by the admin

istration of quinine or procaine amide It is said that procaine amide may cause blood dyscrasia

G Recovery from Muscular Dystrophy

Occasional cases of complete, or almost complete, recovery from muscular dystrophy have been reported (e.g Erb 1908) Nattrass (1954) has reviewed the situation. In reporting eight personal cases he concluded that two were probably examples of benign congenital hypotonia (q v) and that the remaining six were really suffering from polymyositis. Whether this is the explanation remains to be seen. Certainly recovery as opposed to arrest, of muscular dystrophy should always be approached critically

IV THE AMYOTONIA CONGENITA SYNDROME

A. Introduction

The term amyotonia congenita has been applied to a condition of muscular weakness and hypotonia occurring in early infancy Soon after Oppenheim's (1900) first description of the clinical picture, it became clear that the complaint was a syndrome, and not a nosological entity The position has been clarified in recent years by the papers of Sandifer (1954) Brandt (1950) and Walton (1956, 1957a, b) The features of the clinical picture are extreme flaccidity and muscular weakness which are generally noted in the first few weeks or months of life. The child is so limp as almost to slip through the hands, it may be placed in extraordinary postures. Poverty of movement is apparent, and sometimes the mother may have noticed a feebleness of intrauterine fetal movements. The diagnosis has to be made from several possible causes, and these fall conveniently into three groups (Walton, 1957b.)

B Classification

1 *Infantile Spinal Muscular Atrophy (Synonym Werdnig-Hoffmann's Disease)*

This is a form of anterior horn cell degeneration leading to progressive muscular wasting weakness, and hypotonia. In one half of all cases, the condition is present at birth, or appears very shortly after In the remainder it occurs by the end of the first year of life. Death ensues in almost all patients within the first 4 years of life. Survival in a severely crippled state for longer periods has been recorded. The diag

nosis is made on the course, respiratory and bulbar involvement, presence of fasciculation, and loss of reflexes. Electromyography and muscle biopsy are helpful. This disease accounts for two-thirds of all cases of the syndrome. No treatment is effective.

2. *Symptomatic Hypotonia*

A wide range of disorders of infancy may cause hypotonia these include affections of muscles, cerebral lesions, peripheral neuropathy and nutritional and skeletal conditions. The recognition of these, and of benign congenital hypotonia, is fully discussed in the papers mentioned above.

3 *Benign Congenital Hypotonia*

This accounts for the smallest group of cases (about 15%) but as either partial or complete recovery is the rule it is rather important to differentiate these cases from those of infantile spinal atrophy The prospects of full recovery appear to depend upon the degree of muscular wasting present at the outset, those with less muscle weakness are more likely to become entirely normal. When recovery is incomplete mild or moderate persistent atrophy and weakness are found in the limb girdles and trunk. The cause is thought to be a benign congenital muscular dystrophy (Turner 1940 1949) but the question is not finally settled. Electromyography and muscle biopsy are again helpful in diagnosis.

The diagnosis of the "floppy infant," as Sandifer (1954) has stated the problem, may be extremely difficult at the outset only time may reveal the cause with certainty

V INFLAMMATORY CONDITIONS

There is no need to discuss pyogenic or granulomatous infections of muscle here nor does toxic myopathy associated with acute or chronic infectious disease fall within the scope of this chapter However a form of virus infection will be mentioned

Infection with Coxsackie Viruses This is the best example of a virus infection in which muscle involvement is a dominant feature. The clinical manifestations include (1) Epidemic myalgia (Synonyms Bornholm Disease, Pleurodynia) It is now clear that the clinical picture of epidemic myalgia may be caused by Coxsackie Type B

istration of quinine or procaine amide. It is said that procaine amide may cause blood dyscrasia.

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Virus infection. The features of the illness include intense pain in the chest, abdomen, neck, shoulders, or back, and fever The affected muscles are tender and the pains are worse on movement. (2) Meningitis. This is a relatively mild meningitis, with a predominantly lymphocytic response in the cerebrospinal fluid. Clinically Coxsackie infections must be distinguished from pleurisy acute abdominal conditions, poliomyelitis, and other forms of meningitis. The diagnosis is made on the isolation of the virus, usually from the stools. It is also necessary to demonstrate a rising titer of neutralizing antibody against the strain isolated since these viruses, like those of the poliomyelitis group may behave as harmless intestinal parasites. Recovery from the infection is complete.

VI. TRAUMATIC AND VASCULAR DISORDERS

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to be certain of the diagnosis before beginning treatment. Some patients respond at once and can be released from medication after a few weeks, without relapse occurring over some years of observation. Our experience has been that the course of the fulminating variety is not influenced by therapy In the present state of knowledge, cortisone, or prednisone, should certainly be tried in proven cases of all types. Otherwise supportive measures, physiotherapy and the treatment of infections as they arise are all that can be provided for the patient's comfort.

7 Chronic Polymyositis

This diagnosis may be aptly applied to the more chronic forms of the syndrome which has been described above. In practice, the diagnosis can only be made after a full appraisal of all the clinical and pathological evidence available.

IX. MYOPATHY ASSOCIATED WITH DISEASE OF THE THYROID GLAND

Various muscle disorders are associated with thyrotoxicosis, including exophthalmic ophthalmoplegia, acute and chronic thyrotoxic myopathy myasthenia gravis, and periodic paralysis. These disorders may co-exist in the same patient for example a few of our cases of exophthalmic ophthalmoplegia also have myasthenia gravis or chronic thyrotoxic myopathy Muscular symptoms also occur in hypothyroidism The whole subject of muscular disorders and thyroid disease has been reviewed by Millikan and Haines (1953)

A. Muscular Disorders in Hyperthyroidism

1 Exophthalmic Ophthalmoplegia

This is the commonest myopathy associated with thyroid disease (Brain and Turnbull, 1938) The relationship is indirect, and the muscular symptoms may develop with or without thyrotoxicosis. Severe cases are encountered especially in patients who have recently been treated surgically or with anti thyroid preparations, for thyrotoxicosis the interval between treatment and onset of the myopathy is commonly a few weeks, but this is not always so. Exophthalmic ophthalmoplegia may accompany severe thyrotoxicosis, but this is rather uncommon in our material The symptoms are due to an edema of the orbital tissues and external ocular muscles. The muscles may be greatly swollen. Microscopically a lymphocytic infiltration is seen.

In the later stages, the muscles tend to become shrunken and fibrosed These changes cannot be due to the thyroid intoxication, even when this is present, they have been ascribed to the activity of the thyroid stimulating hormone (TSH) of the anterior pituitary gland.

As the name implies, the symptoms are exophthalmos and paralysis of the external ocular muscles, with resultant diplopia. The exophthalmos varies greatly in severity it may be gross, with chemosis and extreme edema of the lids In such cases eye closure may be precluded, so that there is grave danger of corneal ulceration and ultimate panophthalmitis. Papilledema may develop, going on to optic atrophy The degree of exophthalmos and severity of muscle weakness are usually related, but not invariably The muscular weakness commonly involves the abductors and elevators of the globe first, diplopia being produced in the appropriate planes. Eventually total external ophthalmoplegia may supervene. The illness is subacute, the history being measured in weeks rather than months. The exophthalmos is accompanied by much local discomfort in most patients.

Treatment of these patients is difficult. Firstly if there is thyroid intoxication it must be treated Secondly attempts are often made to try to diminish the production of TSH in the pituitary gland by X ray irradiation of the gland or the administration of estrogens or thyroid extract. These measures sometimes appear to be helpful Thirdly local measures are directed towards the preservation of sight if sight is threatened by reason of increasing exophthalmos, orbital decompression is required, and tarsorrhaphy may be useful as an interim measure to prevent corneal ulceration. Inability to close the eyelids is a clear indication for early surgical treatment of this sort. Irradiation of the orbits has been tried with the idea of reducing the edema, but it has not been very successful in our hands. Orally administered cortisone is ineffective Altogether treatment is disappointing but it should be possible to save sight in all cases. The prognosis for full recovery is bad some residual degree of exophthalmos and ophthalmoplegia is the rule Once the complaint is inactive various operations can be performed on the external ocular muscles to overcome residual diplopia.

2. *Thyrotoxic Myopathy*

Thyrotoxic patients commonly complain of some degree of general muscular weakness or fatiguability but more specific muscular disturbances occur

histological changes in the muscles have so far proved to be inconstant and nonspecific. The disorder of function is presumably either at the motor end plate or in the muscle, or at both sites it is thought to be biochemical. Croft (1957) has described the abnormal reaction of some of these patients to the use of muscle relaxants. So far, biochemical studies have not revealed any clues as to the cause of the condition. Bourne and Beckett (q v) have investigated the status of certain muscle enzymes in some of our cases.

Walton (1956) regards these cases as examples of polymyositis. Clinically there are differences between the two syndromes and pathologically we have as yet found no evidence of an inflammatory reaction in the muscles examined. In the circumstances, the noncommittal term myopathy is preferable. Carcinomatous myopathy is an important cause of the proximal muscular syndrome, and, in our experience, a relatively common one The diagnosis may only be confirmed after long observation.

XI. DISORDERS OF EXCITATION AND CONTRACTILITY

A. The Myasthenias

While myasthenia is commonly a symptom of myasthenia gravis, the phenomenon is encountered in other disease states. For example, myasthenic weakness which may respond to the exhibition of neostigmine has been described in carcinomatous myopathy Denny Brown (1947) drew attention to the occurrence of syndromes with myasthenic features in persons suffering from prolonged dietary deficiencies The symptoms might respond to dietetic treatment or, in one group to neostigmine (Katz, 1946) During World War II various tobacco-chewing inhabitants of the Cap Finisterre peninsula developed myasthenic symptoms which were relieved by neostigmine (Coulonjou and Salaun, 1952) When normal supplies of tobacco became available after the war the illness disappeared. This form of myasthenia was thought to be due to the effects of contamination of tobacco with *Clostridium perfringens* Myasthenic weakness has also been reported in dermatomyositis and polymyositis, although the position here is confused by the fact that Stortebecker (1955) has described histological changes in patients with apparently uncomplicated myasthenia gravis which are indistinguishable from those described in polymyositis. Lastly rare patients with clinical pictures resembling either muscular dys-

trophy or benign congenital myopathy have been reported as showing a positive response to neostigmine (Rowland and Eskenazi, 1956 Walton *et al.*, 1956)

B Myasthenia Gravis

Myasthenia is a chronic condition with a tendency to remission and relapse. The myasthenia may be accompanied by permanent muscular atrophy and weakness, especially in longstanding cases. Current opinion inclines to the view that myasthenia gravis is not a disease entity The cause, or causes, remain unknown. The syndrome occurs most commonly between the ages of 20 and 50 but onset may be at any age Females are more affected than males. A thymic tumor is present in about 10% of cases the association with thyrotoxicosis has already been mentioned. The myasthenia is due to some disorder of conduction at the neuromuscular junction in voluntary muscle several explanations have been advanced as to the nature of the lesion, but the problem remains unresolved Histological examination of the muscles, however shows pathological changes (Russell 1953 Stortebecker 1955) and this is not surprising in view of the permanent weakness which may occur These pathological changes also affect the myocardium Russell (1953) emphasizes that none of the changes are specific to myasthenia gravis in her material there was a fair correlation between the extent of the histological changes and the degree of weakness. Electromyography also affords evidence of a muscular lesion (Simpson 1956)

The muscular fatiguability is usually noted first in the external ocular muscles, where it results in ptosis and diplopia. Sometimes the bulbar muscles are affected initially in which case dysarthria and dysphagia are the presenting symptoms. If the process is generalized, the upper limbs tend to be more involved than the lower The facial muscles are frequently affected. Whatever the symptoms, they characteristically appear towards the end of the day disappearing after a night's rest. Eventually permanent weakness develops, and wasting may become manifest. In such cases, fasciculation may be seen. Myasthenic crises occur in some patients, there being a rapid onset of widespread muscular involvement, with grave danger to life from respiratory failure and bulbar paralysis. Exceptionally permanent muscular weakness may be the dominant feature from the outset, so that the patient is thought to have an atypical form of muscular dystrophy

The course of myasthenia gravis is variable. The condition may be

fatal in a few weeks, or on the other hand, it may persist for a lifetime. Prognosis for useful survival is best when the affection is limited to the eye muscles, but untreated cases with severe, generalized weakness may remit for many years. Taking the overall picture, however there is a reduction in the expectation of life and much chronic disability.

Diagnosis rests on the demonstration of weakness which responds to the intravenous injection of edrophonium chloride or to intramuscular administration of neostigmine methyl sulfate. In long standing cases, and in patients with permanent weakness, the response to these drugs may be reduced or abolished. The separation of myasthenia gravis from the other myasthenias raises many questions which cannot be explored here.

Treatment may be medical or surgical. The myasthenia may be controlled by oral administration of neostigmine bromide or pyridostygmine. Special methods of management are required when respiratory failure and bulbar palsy threaten. The treatment of myasthenic crises has recently been discussed by Tether (1955). Surgical treatment consists in thymectomy and conflicting reports have been published on its value. For a detailed appraisal of the situation, reference may be made to the recent papers of Eaton and Clagett (1955) and Simpson (1956). The presence of a thymic tumor raises its own problems when such growths are malignant, the prognosis is bad.

C. Periodic Paralysis

Periodic paralysis is a syndrome which may be brought about in different ways.

1. *Familial Periodic Paralysis*

a. Symptoms. This is a hereditary condition. Although it is usually familial, sporadic cases are encountered with identical clinical and biochemical features. The onset is commonly in childhood or early adult life. The patient suffers recurrent attacks of flaccid muscular weakness. These episodes of weakness are liable to be present on waking; they are often provoked by a large meal with a high carbohydrate content, exposure to cold, high fluid intake, or heavy exertion. The weakness affects the limbs and trunk, generally sparing the bulbar and respiratory muscles. It may be mild, or there may be complete paralysis. The distribution can be asymmetrical, and one limb may be

affected alone. In one personal case, all four limbs were involved, but invariably the left more than right. Muscle tone is reduced or lost, according to the severity of the attack, and the tendon reflexes are lost or abolished. The patient with a severe attack presents a dramatic picture, lying motionless upon his bed. Sensation is unaffected. The somatic weakness may lead to difficulty in micturition or defecation, although smooth muscle is spared. If the attack is associated with a low serum potassium, changes are found in the electrocardiogram. Episodes vary in severity and duration in the same patient. Commonly, recovery is complete in a few hours, but sometimes several days elapse before there is full restoration of power Death has been recorded in an attack, but this is excessively rare. Permanent muscular atrophy and weakness form an unusual but well recognized complication the site of the lesion is in the muscle itself, as might be expected.

Periodic paralysis must be differentiated from hysteria. In severe attacks, the distinction is easy the abolition of tendon reflexes and loss of muscular response to electrical stimuli are helpful points. When the weakness is mild, the problem becomes more difficult, even when the diagnosis of periodic paralysis has been previously made In susceptible persons, attacks may be induced by the administration of insulin and glucose, adrenalin, or fluorohydrocortisone

b The Relationship of Potassium to the Paralysis Biemond and Daniels (1934) noted that the serum potassium was lowered in patients with familial periodic paralysis later Aitken *et al.* (1937) both confirmed this and showed that attacks could be terminated by giving potassium chloride. However it has been shown repeatedly that during attacks there is a marked positive potassium balance, the urinary potassium excretion falling to very low levels as the concentration in the plasma falls (Allott and McArdle, 1938, and others) It is believed that the potassium moves from the extracellular fluid into the cells (Allott and McArdle 1938 Danowski *et al.* 1948) While it is theoretically possible that a prior potassium depletion by losses from the renal or gastrointestinal tracts may take place, it is highly unlikely that this could be an important factor in the causation of the attacks (McArdle, 1956) It is well recognized that in experimentally produced hypokalemia in normal subjects, the serum potassium may be reduced to levels below those associated with paralysis in the familial syndrome without the production of weakness. Furthermore, there is ample evidence that in

other hypokalemic states, severe potassium depletion is required before paralysis occurs (Milne *et al.*, 1952 and others) All these observations make it clear that the problem of the familial syndrome with lowered serum potassium is not a simple one of potassium depletion. Incidentally, there is no exact relationship between the level of the serum potassium and the severity of the attack.

Tyler *et al.* (1951) reported a family in whom attacks of periodic paralysis occurred without any significant change in the serum potassium. The attacks did not respond to the administration of potassium salts. Other workers have had similar experiences. Sporadic cases of this type are also encountered.

c. Theories as to Causation. McArdle (1956) has reviewed the problem of causation in the familial syndrome with low serum potassium. He recalls that since localized attacks can be induced in susceptible persons without lowering of the serum potassium, the muscles them selves must be at fault. This supports the view that the fall in the serum potassium is only one factor in the production of paralysis some other defect in conduction of the impulse along the muscle fiber must also be present. McArdle suggests that a disturbance of membrane permea bility could conceivably result in periodic paralysis by interfering with either active or passive transport of potassium. On the other hand, the abnormal shift of potassium into the cells might be due to an error of carbohydrate metabolism. There is some evidence of increased carbohydrate tolerance in periodic paralysis, indicating overactive glucose storage, and the potassium migration and paralysis might be secondary manifestations of this (MacGregor and Shaper 1957) Allott and McArdle (1938) have previously suggested that there might be some disturbance of the enzyme systems concerned in carbohydrate metabolism at the hexose phosphate level. Conn *et al* (1957) have broken new ground in the investigation of the problem. From their observations on two patients with the familial low serum potassium syndrome, they report massive retention of sodium and increased urinary excretion of aldosterone before attacks of paralysis. Recovery was associated with sodium diuresis. The sodium content of muscle was increased. They also observed the vacuolation of muscle fibers reported in biopsy specimens by Tyler *et al.* (1951) in their series of patients with normal serum potassium. Conn and his co-workers suggest that an abnormally high concentration of sodium within the

muscle is partly responsible for the paralysis this concentration is due to the activity of a sodium retaining corticoid. They claim that a low sodium diet is of prophylactic value. Clearly knowledge of the funda mental disorders concerned in the production of the syndrome is defective.

d. Prognosis and Treatment The attacks tend to become slighter and ultimately to disappear in later life. Prophylactic treatment consists in avoiding things likely to precipitate an attack, such as over-exertion, exposure to cold, and excessive carbohydrate intake. The possible value of a low sodium diet has been mentioned. In attacks associated with low serum potassium, 10 g of potassium chloride should be given by mouth at the outset. When attacks are frequent, daily supplements of potassium chloride may be given. In rare cases with respiratory failure, special methods of artificial respiration may be required

2. *Muscle Disorders Associated with Other Disturbances of Potassium Metabolism*

Instances of muscular weakness have been reported in several disease states in association with a low serum potassium Hyperkalemia can also produce a widespread flaccid paralysis which may be reversed by appropriate treatment.

a. Hypokalemia. Excessive potassium loss is encountered in some forms of nephritis and in the de Toni Fanconi syndrome. Renal loss of potassium with sodium retention takes place as one of the body's responses to surgical operations. In hypokalemia due to these or other mechanisms, electrocardiographic changes form the commonest index of a muscular lesion muscular weakness is rarely a prominent feature of the clinical picture, but examples of periodic or more permanent paralysis are seen, especially in renal failure with loss of potassium. Rapid reversal of the paralysis is achieved by the administration of potassium chloride, but this must be cautiously carried out, particularly in patients with renal disease.

b. Primary Aldosteronism Conn (1955) has drawn attention to the loss of potassium and retention of sodium which follows excessive produc tion of aldosterone in the adrenal cortex, usually in association with an adrenal cortical tumor A patient of Milne *et al* (1957) with such a

tumor suffered attacks of periodic paralysis at 3-month intervals for 9 years. The episodes lasted from 2 to 20 days. Between the attacks, there was no muscular weakness. The serum potassium was low during episodes, when the electrocardiogram showed the changes associated with potassium deficiency. Potassium supplements relieved the symptoms. The differential diagnosis of potassium losing nephritis and primary aldosteronism can be extremely difficult.

c *Hyperkalemia.* Potassium intoxication occurs in salt losing nephritis, acute renal cortical ischemia (as in the crush syndrome, mismatched blood transfusions, or after abortion) and in adrenal failure. The electrocardiogram becomes abnormal as the serum potassium rises. Occasionally potassium poisoning usually due to renal disease, causes the rapid onset of a flaccid paralysis which may be of ascending type. In these patients, the tendon reflexes are lost, and respiratory failure and bulbar palsy may supervene. The muscles respond to electrical or even mechanical stimulation. Death results from cardiac arrest. Commonly however patients show nothing more than general muscular enfeeblement, perhaps with reduction of reflexes indeed, death from cardiac arrest occurs without the appearance of remarkable muscle weakness. From the practical aspect, the following points emerge (1) hyperkalemia due to renal disease must be considered a rare cause of acute flaccid paralysis (2) adrenal failure may present with muscular weakness as a prominent symptom. Treatment consists in restoring the electrolyte balance by methods appropriate to the underlying disease process.

3 *Periodic Paralysis Associated with Thyrotoxicosis*

Brain (1955) states that symptoms indistinguishable from those of periodic paralysis have been reported in a few patients in association with thyrotoxicosis, he has had one example. The symptoms disappear after thyroidectomy

Studies on the histochemistry of muscle in most of the above diseases are described in Chapter I Volume III

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CHAPTER XII

Genetic Aspects of Muscular and Neuromuscular Diseases

JOHN N. WALTON

I INTRODUCTION

The last 50 years have seen great advances in our understanding of the complexities of human inheritance. Among the many human diseases which can be directly attributed to genetic influence are several disorders of the muscular and neuromuscular systems. In many other similar conditions, there is some evidence that inherited characters have a less powerful but nevertheless significant effect upon the development of the disease process. Before describing the mode of inheritance of individual diseases, however, it is important to consider some general principles of human genetics.

II. SOME PRINCIPLES OF HUMAN GENETICS

The science of human genetics is concerned with the "inborn characteristics of man and with the qualities which distinguish man from other species and one human individual from another Its study embraces not only a consideration of the means of transmission of inherited characteristics from one generation to the next but also seeks to determine the way in which these characters produce their effects. Whereas some congenital abnormalities are clearly inherited so too are many diseases which develop late in life we therefore need to recognize not only the relationship between the inherited character and disease, but also the nature of the dynamic process, partly prenatal and partly postnatal, through which it acts. The practical value of such information is clear the example of diabetes has shown that a disorder in which genetic influences are important can nevertheless be treated effectively *Many hereditary diseases which are at present incurable* may be controlled in the future, once it proves possible to correct by biochemical or other means the mechanism by which the inherited character produces its effect upon the human organism.

Although man, with his infinitely diverse genetic constitution and environment, and his random matings, is in general an unsatisfactory subject for genetic study it is often possible to select groups of broadly similar constitution and environment, or marriages, which happen to be appropriate to the particular problem under consideration. While the single family is usually too small a unit to give statistically valid data, pooling of information obtained from a number of similar families may be adequate. By such means, it may be possible to determine with reasonable accuracy the mode of inheritance of a particular disease, while surveys of large numbers of cases of the disease may reveal the incidence and effect of the condition in a particular population. Information of this nature is invaluable not only to the public health worker but also to the individual clinician who may be called upon to advise a patient concerning the advisability of marriage or procreation.

A. Genetic Constitution of the Individual

It has long been believed that the nucleus of every cell in the human body contains 48 chromosomes in 24 pairs. However the recent careful chromosome counts carried out by Tjio and Levan (1956) and by Ford and Hamerton (1956) on cells in tissue culture indicates that in

fact there are only 46 (23 pairs) The fertilized ovum or zygote contains 23 chromosomes derived from the male gamete (spermatozoon) and 23 from the female gamete (ovum) it subsequently divides by mitosis so that each pair of chromosomes in every cell of the human body consists of one derived from the father and one from the mother In 22 of these pairs of chromosomes, each member is similar to the other· in the female, the same is true of the 23rd pair, the sex chromosomes, which are conventionally designated XX In the male, however, whose constitution is XY, one of the sex chromosomes (Y) is much shorter than the other (X) The remaining 22 pairs, which play no part in sex determination, are called the autosomes.

The factors responsible for the transmission of inherited characters, the genes, are arranged in linear order on the individual chromosomes. As each individual possesses a pair of similar chromosomes, and as each chromosome has a series of loci at which genes are situated, it follows that genes, too occur in pairs each being accompanied by a companion gene at the same locus of the homologous chromosome. The two partner genes are called allelomorphs or alleles. The total number of genetic loci in man is unknown, but Stern (1949) gives reasons for suggesting that the figure is probably between 5 000 and 12,000

B. Genic Action

In the physical sense genes are relatively stable in chemical constitution they control the processes of cellular metabolism and influence development through chemical interaction with nongenic material of the cell. It is this type of activity on the part of a gene which so influences developmental processes that normal traits, congenital defects, or diseases are produced. However it should be remembered that this process may be modified by environment. Although the individual's genetic constitution or genotype is determined and immutable from the moment of conception, his observed characteristics or phenotype may result from modification of the genotype by environmental processes. Variations in the effect of a particular gene in different individuals can be called variability in specificity Penetrance, a statistical concept, is a measurement of the frequency with which the effect of gene or genotype is manifest in those who carry the gene or genes in question. Expressivity is an assessment of the degree or severity of the effects of the gene in a particular individual. While these are terms in common use in clinical genetics, many workers prefer to use

the inclusive term manifestation (e.g complete manifestation, incomplete manifestation)

One difficulty in human genetics is that it may sometimes be impossible to determine to what extent a particular disease results from genetic or environmental influences in tuberculosis, for instance, it may be difficult to decide how much of the increased incidence of the disease in a particular family is due to inherited susceptibility and how much to dissemination of infection. Until we have some accurate means of determining genotype, our conclusions concerning the inheritance of disease must be based upon phenotypical observations. Not only will environment modify the picture in certain cases, but it is also apparent that comparatively few genes have single and independent effects. Some may have several different chemical actions some may act in combination with other genes in adjacent loci, any one of which can modify the eventual result. When a particular trait depends entirely upon the interaction of two alleles, this is known as single factor inheritance· variability in expression may then depend upon the modifying effect of one allele upon the other quite apart from environmental influences. Conditions produced by the interaction of many different genes are said to be subject to multifactor inheritance, and this can be very complex.

It is fortunate for the geneticist that many genes produce their effects independent of environment, those responsible for the human blood groups, and which occur as multiple alleles are examples. When two alleles are identical, the individual is said to be homozygous when they are different, he is heterozygous Some genes produce an effect independent of the nature of the other allele and are then said to show complete dominance in other individuals, two identical alleles must be present for the phenotypical effect to appear and this gene is said to show complete recessivity It is conventional to indicate a dominant gene with a capital letter and a recessive gene with a small letter Hence we may have the combination Aa, in which case the dominant gene A produces the phenotype (expression in the heterozygote) while the individual is a carrier of the recessive gene a, the latter producing no phenotypical effect. In complete dominance, such a person cannot be distinguished from one with the constitution AA the individual aa will show the recessive trait (expression only in the homozygote) While these illustrations typify complete dominance and complete recessivity respectively variable effects may be produced by

interaction of the alleles, thus certain heterozygous carriers of recessive genes can be identified by minor clinical or biochemical abnormalities. The great majority of genetically determined diseases are caused by genes belonging to simple allelic systems, and most of them are carried on the autosomes and are therefore known as autosomal dominant or autosomal recessive characters. There are, however a small but important group of disorders due to recessive genes which lie upon the differential (unpaired) portion of the X chromosome. Despite their recessivity these genes are invariably expressed in the male as there is no dominant allele to suppress their effects in the female, who has two X chromosomes, however the condition is only evident in the homozygote. This type of gene, as it is transmitted on a sex-determining chromosome, is called a sex-linked recessive.

Before going on to describe the forms of inheritance which are encountered in human genetics, it is important to mention briefly linkage, which can sometimes explain the occurrence of multiple defects in a family and mutation, which may be the reason for the appearance of a disease in a family in which it was previously unknown.

C. Linkage and Crossing Over

During the process of meiosis or cell division in the ovary or testis a process which results in the production of mature gametes, the chromosomes derived from the mother and father of the individual concerned join in pairs and each chromosome then becomes double. At this stage an exchange of homologous segments of adjacent chromosomes, known as "crossing over" may occur Supposing that meiosis results in the production of four gametes, each containing a single chromosome then one will probably contain a chromosome derived from the father another will probably contain a chromosome derived from the mother while the other two may contain chromosomes which are hybrids, each containing a segment derived from the paternal and maternal chromosomes. Supposing two human genes are located in different chromosomes, they will be transmitted independently but if they lie on the same chromosome they will be passed on together unless crossing over has occurred during formation of the gamete. If they lie very close together they may be said to be linked, and will generally cross over together Hence if two genetically determined defects occur in a family the frequency with which the two occur in

carrier is 1 in 100 hence we would expect 1 in every 100 affected individuals to have an affected child. Clearly the probability of such an event would be greatly increased by a consanguineous marriage.

a. Consanguinity If an individual who is heterozygous for a recessive gene marries a first cousin, then it is apparent that the chance of his spouse being heterozygous for the same gene is 1 in 8, irrespective of the incidence of the gene in the population. It is thus clear that marriages between blood relations are likely to result in the expression of recessive genes which may have been carried unsuspected by members of the family for generations. Conversely when a genetic disease appears in a sibship resulting from a consanguineous union, then the condition is in all probability due to a recessive gene rather than a mutation. Certain very rare disorders due to recessive genes may have an incidence of almost zero in the general population, whereas in isolated communities where consanguineous marriage is common, they may be relatively frequent.

The frequency of consanguineous marriages varies greatly in different parts of the world, being affected by a great many social geographical and religious factors. Bell (1933) found an incidence of 0 6% first cousin marriages in a group of patients seen in general hospitals in England, while in Germany Lenz (1938) found an incidence of 1% Böök (1948) reported a frequency of 0.95 ± 0.32% in a North Swedish population, while in a South Swedish agricultural community the incidence according to Larson (1956) was 1 7% In certain isolated areas of Switzerland, Hanhart (1923) found when studying families with Friedreich s ataxia that the incidence was as high as 17%

3 *Sex Linked Recessive Inheritance*

It has already been mentioned that a recessive gene which lies on the differential (unpaired) segment of the X chromosome will invariably be expressed in the male as there is no allele to suppress its effect. Hence, all completely sex linked recessive conditions, of which hemophilia and color blindness are examples, are very much more common in the male than in the female since the female must be homozygous, carrying the recessive gene on each X chromosome, for the disease to appear Furthermore all males carrying the gene will show the defect, so that if an affected male s father is unaffected, he must have received

the condition from his mother a heterozygous carrier Characteristically therefore, sex-linked disorders are manifest in males and transmitted by females. As the carrier female will pass on each of her X chromosomes, only one of which carries the gene, to half of her sons and daughters, half of her sons will show the defect and half will be normal, while half of her daughters will be carriers, who can in turn pass on the condition. In other words, the ratio of affected unaffected males and of carrier normal females in such a sibship will be 1 1

A certain number of very rare conditions are due to genes which are carried on the paired portions of the X and Y chromosomes (partial sex linkage) or on the very small differential portion of the Y chromosome (Y linkage) but these are of little practical importance in clinical genetics.

4 *Sex Limited Inheritance*

For reasons which are not fully understood, certain autosomal genes, either dominant or recessive, are expressed only in one sex. The mechanism of this sex-limitation is far from clear of practical importance is the fact that a disease due to a sex limited gene which produces its effects only in males may be very difficult to distinguish from a sex linked character

F The Methodology of Human Genetics

Since the number of children in a sibship is often comparatively small, information derived from a single family is rarely sufficient for accurate conclusions to be drawn concerning the mode of inheritance of a disease or congenital defect. Hence information from a number of families must be pooled before the findings can be statistically valid. Whenever possible, it is also important to discover all the cases of a particular disease within a defined geographical region of known total population. With such complete ascertainment of cases it is possible to calculate not only the incidence of the disease in the population but also the gene frequency and possibly the mutation rate of the gene. However conclusions concerning the mode of inheritance of any individual gene must be drawn from the study of families or kindreds in which the gene is occurring while valuable information concerning the effects of the gene can also be derived from detailed consideration of twin pairs which happen to occur within these families.

1 *The Study of Families*

Families are usually ascertained first by the discovery of affected individuals, known as propositi or probands. From these propositi or initial cases it may then be possible to construct a family tree, including details of all affected and unaffected individuals. Sometimes information obtained concerning the parents and sibs of the propositi may be adequate but often it is necessary to delve more deeply. An important difficulty is the fact that uniformity of a clinical picture in two individuals does not necessarily imply that they are of identical genotype, while conversely the same genotype may produce diverse effects in different individuals owing to modification by environment and other factors. It is thus important not only to study ratios between affected and unaffected individuals, in an attempt to define the mode of inheritance of a character but also to find some means of identifying individual genotypes so that the reasons why a particular gene may affect one individual in a certain way and another differently may be deduced. From this type of study we may be able to discover exogenous methods of modifying the effects of noxious genes. It cannot be stressed too strongly that in studies of this nature it is important that ascertainment should be complete i.e. all members of the family both affected and unaffected, should be examined personally, not only by all available clinical means but also utilizing appropriate biochemical techniques wherever possible. Not only do certain diseases present in so mild a form that slightly affected individuals and their relatives may be unaware that they are affected but in other instances heterozygous carriers of a recessive gene may exhibit minor recognizable effects of the gene. The age of onset and duration of the disease must always be borne in mind in investigations of this type for if the parent of an affected individual should die at an age earlier than that at which the disease generally develops, one may falsely assume that the disease has "skipped" a generation.

It may also be valuable in certain families to examine members for evidence of other genetic traits whose mode of inheritance is fully understood, as it may then be possible to demonstrate linkage or crossing over between one of these genes and that responsible for the disease under consideration. Information of this nature will make it possible to map out the loci of individual disease genes upon the human chromosomes. Furthermore, such family studies can have the incidental effect of delineating accurately the natural history of the disease and

its relationship to other disorders of similar clinical presentation some conditions may be found to show the phenomenon of anticipation, in which the disease appears at an earlier age and with increasing severity in successive generations. Information of this type can be of great value to the clinician.

In attempting to calculate ratios of affected to unaffected individuals in order to decide upon the mechanism of inheritance of a particular gene, it should be remembered that families discovered in this way almost invariably constitute a highly selected group containing a preponderance of affected individuals. For instance, if the gene is a recessive, there will be many small families produced by the mating of Aa and Aa which will contain no affected individuals and will not therefore be ascertained. In an attempt to overcome this bias, several statistical devices are in use, of which the most popular is Weinberg's proband method with this technique, the propositi are excluded from the calculation and the ratio of affected to unaffected individuals is based upon the observed incidence in the sibs of the propositi.

2. *Twin Studies*

Monozygotic twins are genotypically identical, while dizygotic twins are no more alike than any pair of sibs. Discordance, or significant difference in phenotype between a pair of monozygotic twins, instead of the expected concordance, may mean either that the gene is of variable expressivity or penetrance or that its effect has been modified by environmental factors. Hence the study of twin pairs can give unique opportunities for studying the penetrance of genes and the interaction of genetic factors and the environment. Unfortunately at least 30 or 40 twin pairs of like sex are necessary for adequate statistical conclusions to be drawn so that the genotype under consideration must of necessity be common. As most of the inherited muscular and neuromuscular diseases are comparatively rare, twin studies give little information of value in the elucidation of their inheritance Hence this subject does not warrant detailed consideration here it has recently been reviewed by Waterhouse (1953)

Having mentioned some of the principles of human genetics and the difficulties which abound in this field it will be convenient to consider the principal muscular and neuromuscular diseases which can be attributed wholly or in part to genetic factors.

III. DISEASES OF MUSCLE

A. Progressive Muscular Dystrophy

The classification of the muscular dystrophies has been a matter of dispute for many years, but the position has been clarified by comprehensive studies carried out in several parts of the world during the last few years. Classically this group of disorders has been divided into the pseudohypertrophic (Duchenne, 1868 Gowers, 1879) pelvic girdle atrophic (Leyden 1876 Möbius, 1879) facio-scapulo-humeral (Landouzy and Dejerine, 1884) juvenile scapulo-humeral (Erb, 1884) distal (Gowers, 1902), late-life (Nevin, 1936) Barnes type (Barnes, 1932) and ocular (Hutchinson, 1879 Fuchs, 1890) forms. Of these the distal, ocular and Barnes types are rare and indeed the latter has only been discovered in a single family· for the present, these three uncommon forms of muscular dystrophy must still be regarded as distinctive entities and will receive separate consideration below Furthermore, it is now apparent that although true muscular dystrophy may occasionally begin in late middle age (Walton and Nattrass, 1954) many cases of so-called late-life or "menopausal" muscular dystrophy (Shy and McEachern, 1951) are suffering from a form of polymyositis, a condition which is not genetically determined and which is different both clinically and pathologically from the inherited forms of dystrophy

We are thus left to consider the pseudohypertrophic, pelvic girdle atrophic, facio-scapulo-humeral, and juvenile scapulo-humeral forms into which the great majority of cases of muscular dystrophy can be classified. This purely clinicoanatomical classification has led to considerable confusion concerning the mode of inheritance of the different varieties of the disease. Thus Sjövall (1936) and Milhorat and Wolff (1943) observed autosomal dominant, recessive and sex-linked patterns of inheritance in dystrophy families, but were unable to identify any single genetic mechanism as being invariable in any of the classic forms of the disease. Similarly Bell (1943) in a monumental survey of *1,228* published cases and of *another 113 gleaned from the* records of the National Hospital, Queen Square, divided the cases into three principal groups which were

A. Pseudohypertrophic all cases with muscular pseudohypertrophy but without muscular involvement.

B Atrophic cases with muscular atrophy but without pseudohypertrophy or facial involvement.

C. All cases with facial involvement.

Although a sex linked recessive inheritance was most common in Group A, an autosomal recessive in Group B and an autosomal dominant in Group C, examples of all three genetic patterns were found in each of the clinical groups. Bell (1943) therefore concluded that the same main gene could give rise to any of the three clinical types and that in this respect muscular dystrophy was a unique disease.

Subsequent workers (Tyler and Wintrobe, 1950 Stevenson, 1953 Becker 1953 Walton and Nattrass, 1954) have however criticized Bell's observations, pointing out that any survey such as hers is likely to perpetuate the errors which were present in many of the reports she reviewed, and indicating that a single clinical feature, such as muscular pseudohypertrophy is an unsatisfactory criterion upon which to base any classification particularly since this phenomenon may occur in all forms of muscular dystrophy and even in other diseases such as familial periodic paralysis (Tyler 1950)

Tyler and Wintrobe (1950) basing their observations on a series of cases observed in Utah, subsequently expressed the view that most cases can be classified into two groups, namely the childhood and facio-scapulo-humeral forms. The childhood form embraced the pseudohypertrophic and atrophic pelvifemoral cases beginning in childhood, while the facio-scapulo-humeral group embraced not only the cases corresponding to the classic descriptions of Landouzy and Dejerine (1884) but also the juvenile scapulohumeral variety The inheritance of the childhood type suggested transmission by a sex-linked recessive gene, while the facio-scapulo-humeral variety was almost invariably an autosomal dominant character

Levison (1951) who reviewed a large series of cases in Denmark, also classified the pseudohypertrophic and atrophic pelvifemoral cases together but unfortunately he did not take account of the age of onset of the disease in these cases, so that in this group he found examples of sex-linked recessive autosomal recessive and autosomal dominant inheritance. However he regarded the juvenile scapulohumeral and facio-scapulo-humeral varieties as distinctive entities, finding that the first was usually the expression of an autosomal recessive the second of an autosomal dominant gene.

Stevenson (1953) reporting his findings in 51 families in Northern

Ireland, and subsequently in a further 9 (Stevenson, 1955) concluded, like Tyler and Wintrobe, that cases could be divided into two main groups. The first variety which he entitled "Duchenne type rapidly progressive muscular dystrophy of young boys," was inherited as a sex linked recessive character while the second, "autosomal limb-girdle muscular dystrophy" which embraced cases of later onset, with or without affected faces, and beginning in either the shoulder or pelvic girdle, included examples of both autosomal recessive and autosomal dominant inheritance.

From a study of 259 cases of muscular dystrophy ascertained in the Freiburg area of Germany during the years 1904–1941 of which 159 were examined personally Becker (1953) concluded that the disease occurred in two principal clinical forms. These were first the shoulder girdle or descending form and secondly the pelvic girdle or ascending form. *The two varieties seemed to be clinically and genetically distinct.* The very mild descending form, which often involved the face, was usually inherited as an autosomal dominant character though a large number of isolated cases occurred. In the pelvic girdle or ascending form, two subgroups could be distinguished. The first subgroup was a rapidly progressive disorder of early onset and *was usually due to a* sex linked recessive gene while the second was more benign of later onset, and usually identifiable as an autosomal recessive character The classification adopted by Hanhart (1954) was similar

While each of these more recent classifications is much more satisfactory than the classic one, Walton and Nattrass (1954) suggested that none of them was entirely compatible with the findings they observed in a series of 84 cases discovered in Northeast England. These authors agreed that the Duchenne type dystrophy was inherited as a sex linked recessive character and that pseudohypertrophy though most common in this form could occur in any form of muscular dystrophy However the term "childhood" as used by Tyler and Wintrobe (1950) was also unsatisfactory as occasional clinically typical cases of this type were of later onset. This fact, combined with the very occasional occurrence of this variety in girls, was sufficient to invalidate Stevenson s (1953) rigid categorization of such cases. They agreed that the remaining cases were inherited by an autosomal mechanism but felt that the cases with affected faces, which were almost invariably due to a dominant gene, should be distinguished from the limb-girdle cases where the onset was in either the pelvic or shoulder girdles and in

which the inheritance was recessive. In other words, the commonly occurring cases of muscular dystrophy could be divided into three groups which were clinically and genetically distinct. These were the Duchenne (sex-linked recessive) facio-scapulo-humeral (autosomal dominant) and limb-girdle (autosomal recessive) forms. It will be seen that this classification agrees broadly with those of Tyler and Wintrobe (1950) Stevenson (1953) and Becker (1953) differing only in detail.

1 *Duchenne Type Muscular Dystrophy*

This form of muscular dystrophy according to Walton and Nattrass (1954), is characterized by (a) expression usually in the male but rarely in the female (b) onset usually in the first 3 years of life but occasionally as late as the third decade (c) transmission as a sex linked recessive character (d) symmetrical involvement first of the pelvic girdle muscles, later of the shoulder girdles (e) pseudohyper trophy particularly of the calf muscles, in about 80% of cases (f) steady and rapid progression leading usually to inability to walk within 10 years of the onset and subsequently producing progressive deform ity with muscular contractures and skeletal distortion and atrophy Death from inanition or respiratory infection occurs often in the second decade, but since the widespread use of antibiotics it has been delayed in many cases until middle life.

Evidence in support of the hypothesis that this form of the disease is transmitted as a sex linked recessive character comes first from an examination of characteristic pedigrees (Fig 1) Although many isolated cases of this type are seen, and in other families the disease is found in the males of a single sibship only there are now a great many families on record (Bell, 1943 Tyler and Stephens 1951 Stevenson 1953 1955 Becker 1953 Walton, 1955 1956a) in which the disease was manifest in males and transmitted by females through several generations. Thus Tyler and Stephens (1951) found 61 cases in 33 families in 7 families, there was clear evidence that carrier females had transmitted the gene to 31 of their 58 male offspring In 14 families, the data were insufficient to determine whether the trait was passed on by carrier females, while in the remaining 12 families, isolated cases occurred In Walton s (1955) series of 54 cases in 34 families 20 were isolated, in 8 families more than one boy was affected in a single sibship while in 6 families the disease had occurred in more than one

generation, being transmitted by females. In the latter series of cases, if the 14 affected individuals with no male sibs were excluded, the incidence of 40 affected and 33 unaffected males approached the 1 : 1 ratio to be expected with a sex linked recessive gene. There seems to be little doubt that the penetrance and expressivity of this gene are complete. No clear evidence of anticipation was found by Bell (1943) in the families she reviewed.

Walton (1955) included in his series of cases two females who were affected by the disease in typical form. As very few affected males with

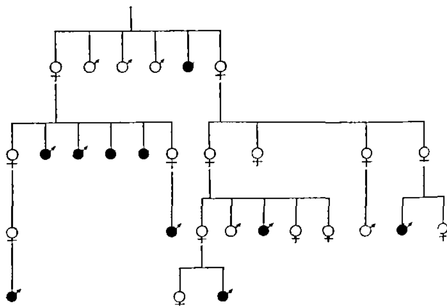


FIG. 1 A pedigree of the Duchenne type muscular dystrophy illustrating sex linked recessive inheritance (from Walton, 1956a)

KEY TO PEDIGREES IN FIGS. 1–18

♂	male	⊗	possibly affected
♀	female	⊘	cataract
○	sex unknown	CB	red-green colour blindness
④	4 individuals, either sex	N	normal colour vision
⊘	stillbirth, or died in infancy	M	mentally defective
●	affected	D	died in childhood or early adult life
◐	partially affected	—⊥—	illegitimate child

this condition reproduce, such an event would have to be explained by the mating of a carrier female with a male upon whose X chromosome a mutation had occurred. It was estimated however (Walton, 1956a) that the ratio affected females affected males would be approximately 1 50,000 so that affected females would be expected to be very rare unless the mutation rate of this gene were very much higher in males than in females. Haldane (1956) has given reasons for suggesting that this may indeed be the case. Hence the occurrence of occasional affected females does not invalidate the sex linked recessive hypothesis.

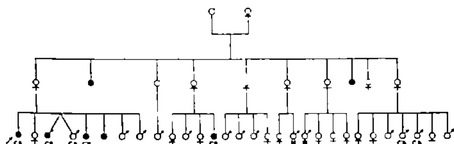


FIG. 2. A pedigree (for key see Fig 1) of the Duchenne type muscular dystrophy illustrating crossing over with incomplete red-green color blindness (from Philip and Walton, 1956)

Another point of interest is that Walton (1956a) found that an affected female," occurring in a family with many affected males, was also a case of gonadal agenesis (Turner's syndrome) and nuclear sexing studies indicated that "her" chromosomal sex was male.

Additional support for the view that this form of muscular dystrophy is due to a sex linked recessive gene comes from the observation that it may occur in children of the same mother but of different fathers (Milhorat and Wolff 1943 Walton, 1955) and from the fact that crossing over with red-green color blindness (Fig 2) has been reported in one family (Philip and Walton, 1956) Smith (1956) confirmed that in this family the crossing over percentage was 25% and that the data supported sex linked inheritance of the muscular disease Despite the impressive evidence in support of this view it must, however be mentioned that Lamy and de Grouchy (1954) and Kloepfer and Talley (1957) have reported families, believed to be of this type in which the condition affected both males and females, and the findings were suggestive of autosomal recessive inheritance. Others would con sider that despite the similarity of the clinical findings in these cases to

those of the Duchenne type, these families would more properly be classified in the limb-girdle group

The fact that so many isolated cases of this form of muscular dystrophy occur (Fig 3) would suggest that many arise as a result of mutation in the mother or in the maternal grandfather This view is supported by the observations of Tyler and Stephens (1951) and Stephens (1953) that in one family the disease occurred in male monozygotic twins while 7 other male sibs were normal Furthermore, Walton (1955) found that in his 34 families, only 6 of 65 maternal uncles at risk were affected in generations earlier than those of the

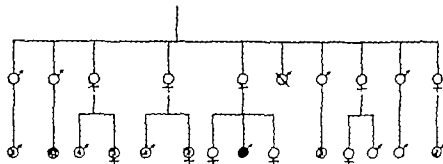


FIG. 3 A case of the Duchenne type muscular dystrophy which was almost certainly the result of mutation (from Walton, 1955) For key to pedigree see Fig. 1

propositi, while in those sibships of propositi containing more than one male, with no cases in previous generations, only 10 male sibs of the propositi were affected, 20 being normal This incidence of 33.3% though higher than the figures of 22.2 and 18.4% respectively reported by Stevenson (1953) and by Tyler and Stephens (1951) falls well short of the expected 50% and gives additional weight to the assumption that many of these boys were the first recipients of a mutant gene.

a. Mutation Rate In view of the fact that very few affected males with this condition reproduce, the method of Haldane (1935) for calculating the mutation rate would seem applicable He suggests that $U = \frac{1}{3}(1-f)x$ where U is the mutation rate per gene per generation, f is the fertility rate of affected males relative to that of males in the general population, and x is the frequency of affected individuals. In this instance $f = 0$, $U = \frac{1}{3}x$ Using this formula, Tyler and Stephens (1951) estimated the mutation rate in Utah to be 9.5×10^{-5} while Stevenson's (1953) estimate for Northern Ireland was initially given as between 4.5 and 6.5×10^{-5} but was later modified (Stevenson, 1955)

to between 5 4 and 7.2 × 10^{-5} Walton (1955) calculated that the rate for Northeastern England was 4.3 × 10^{-5} These rates are some of the highest yet recorded in human genetics.

2 *Facio-Scapulo-Humeral Muscular Dystrophy*

Walton and Nattrass (1954) concluded that the characteristics of this form of the disease were (a) expression in either sex (b) onset at any age from childhood until late adult life (c) transmission usually as an autosomal dominant character (d) abortive or mildly affected cases are common (e) involvement first of the face and shoulder

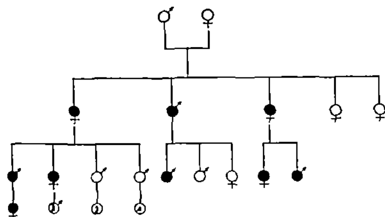


Fig. 4 A pedigree (for key see Fig. 1) of facio-scapulo-humeral muscular dystrophy illustrating autosomal dominant inheritance (from Walton, 1955)

girdle muscles with subsequent spread to the pelvic girdle (f) muscular pseudohypertrophy is very uncommon (g) progress of the disease is insidious with prolonged periods of arrest and most patients survive and remain active to a normal age

In most reported families, this form of the disease has been inherited as an autosomal dominant character (Fig 4) (Bell 1943 Boyes *et al* 1949 Tyler and Stephens, 1950 Becker 1953 Walton 1955 1956a.) It must be stressed that in this form of muscular dystrophy above all others, examination of all available relatives is essential in any genetic study as the mildly affected or abortive cases may be totally unaware, as are their relatives, that they are suffering from the disease in a mild form. Thus hearsay evidence can give a totally inaccurate impression. It is also important that examination should be carried out by experienced individuals, as minimal facial weakness, for instance, may be

easily missed. However it is apparent that Stevenson (1953) was aware of these pitfalls, and in certain of his families in Northern Ireland the disease appeared to be due to an autosomal recessive gene.

The very large family reported by Tyler and Stephens (1950) gives excellent opportunities for studying the inheritance of this condition. Of 1249 individuals in 6 generations, 159 were affected, and all affected individuals had affected parents in no case had an unaffected individual passed on the disease to his offspring. Taking into account only those sibships about which accurate information was available, and excluding all children less than 12 years of age, there were 150 affected

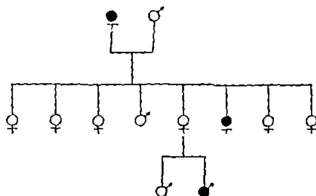


FIG. 5. A pedigree (for key see Fig 1) of facio-scapulo-humeral muscular dystrophy suggesting incomplete penetrance of the gene (from Walton, 1955)

and 143 unaffected individuals. Hence in this family it is apparent that penetrance of the gene was complete. The expression of the gene varied however as some few individuals were severely affected many more very slightly indeed. It seemed improbable that environment could have been responsible for this degree of variability and an effect of modifying genes (possibly the allele) was a more plausible cause of clinical variability No evidence of anticipation was discovered in this family

A similar modifying effect of the allele may have been responsible for the apparent incomplete penetrance exhibited by the gene (Fig 5) in certain of the families reported by Walton (1955 1956a) Occasionally an individual who was apparently unaffected had passed on the disease unfortunately some such parents were not available for examination certain families also showed apparent sex-limitation (to females) but yet the gene was clearly dominant in its effects (Fig 6)

It may be that similar modification of the effects of a dominant gene was responsible for the impression of recessivity gained by Stevenson (1953) in some of his families.

Attempts to demonstrate linkage between the gene for this condition and those responsible for the blood groups, for the ability to taste phenylthiocarbamide, or for the secretion of A and B substances in the saliva have been made by many workers (Tyler and Stephens, 1950 Walton, 1955 Race, 1955 Stevenson *et al.*, 1955) but to date no convincing or even suggestive evidence of linkage has been obtained

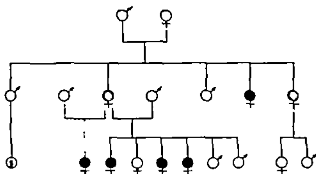


FIG. 6. A family of facio-scapulo-humeral muscular dystrophy in which there appeared to be sex-limitation (from Walton, 1955) For key to pedigree see Fig 1

3 *Limb-Girdle Muscular Dystrophy*

The features of this variety of muscular dystrophy as defined by Walton and Nattrass (1954) are (a) expression in either sex (b) onset usually late in the first or in the second or third decades but occasionally in middle age (c) transmission as an autosomal recessive character (d) primary involvement of either the shoulder girdle muscles or of the pelvic girdle with spread to the other after a variable period (e) muscular pseudohypertrophy is uncommon (f) abortive cases are uncommon (g) variable severity and rate of progression, intermediate between those of the Duchenne and facio-scapulo-humeral forms (h) most patients become severely disabled in middle life and die before the normal age

A great many cases of this type are isolated but often more than one member of a sibship is affected (Fig 7) without there having been any cases of the disease in previous generations (Bell, 1943 Stevenson 1953 Becker 1953 Walton, 1955 1956a) This pattern is of course

easily missed. However it is apparent that Stevenson (1953) was aware of these pitfalls, and in certain of his families in Northern Ireland the disease appeared to be due to an autosomal recessive gene

The very large family reported by Tyler and Stephens (1950) gives excellent opportunities for studying the inheritance of this condition. Of 1249 individuals in 6 generations, 159 were affected and all affected individuals had affected parents in no case had an unaffected individual passed on the disease to his offspring Taking into account only those sibships about which accurate information was available, and excluding all children less than 12 years of age, there were 130 affected

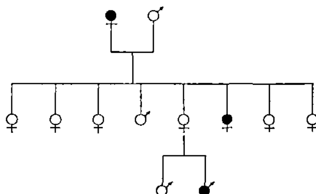


FIG. 5 A pedigree (for key see Fig. 1) of facio-scapulo-humeral muscular dystrophy suggesting incomplete penetrance of the gene (from Walton, 1955)

and 143 unaffected individuals. Hence in this family it is apparent that penetrance of the gene was complete. The expression of the gene varied however as some few individuals were severely affected, many more very slightly indeed. It seemed improbable that environment could have been responsible for this degree of variability and an effect of modifying genes (possibly the allele) was a more plausible cause of clinical variability No evidence of anticipation was discovered in this family

A similar modifying effect of the allele may have been responsible for the apparent incomplete penetrance exhibited by the gene (Fig 5) in certain of the families reported by Walton (1955 1956a) Occasionally an individual who was apparently unaffected had passed on the disease unfortunately some such parents were not available for examination certain families also showed apparent sex limitation (to females) but yet the gene was clearly dominant in its effects (Fig 6)

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Attempts to demonstrate linkage between the gene for this condition and those responsible for the blood groups, for the ability to taste phenylthiocarbamide, or for the secretion of A and B substances in the saliva have been made by many workers (Tyler and Stephens, 1950 Walton, 1955 Race, 1955 Stevenson *et al.*, 1955) but to date no convincing or even suggestive evidence of linkage has been obtained

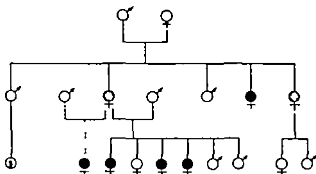


FIG. 6. A family of facio-scapulo-humeral muscular dystrophy in which there appeared to be sex-limitation (from Walton, 1955) For key to pedigree see Fig 1

3 *Limb-Girdle Muscular Dystrophy*

The features of this variety of muscular dystrophy as defined by Walton and Nattrass (1954) are (a) expression in either sex (b) onset usually late in the first or in the second or third decades, but occasionally in middle age (c) transmission as an autosomal recessive character (d) primary involvement of either the shoulder girdle muscles or of the pelvic girdle with spread to the other after a variable period (e) muscular pseudohypertrophy is uncommon (f) abortive cases are uncommon (g) variable severity and rate of progression intermediate between those of the Duchenne and facio-scapulo-humeral forms (h) most patients become severely disabled in middle life and die before the normal age.

A great many cases of this type are isolated, but often more than one member of a sibship is affected (Fig 7) without there having been any cases of the disease in previous generations (Bell, 1943 Stevenson, 1953 Becker 1953 Walton 1955 1956a) This pattern is of course

suggestive of autosomal recessive inheritance. This conclusion is strengthened by the fact that this form of the disease is particularly common in Switzerland where consanguinity is frequent (Hanhart, 1954) Affected individuals were the products of consanguineous marriages in 5 of 23 families of this type reported by Stevenson (1953) and in 1 of the 18 families described by Walton (1955) In Walton's (1955) series, the incidence of 20 affected and 51 unaffected individuals

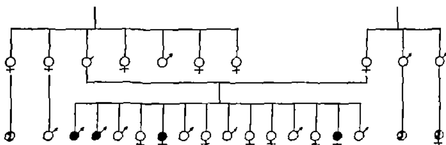


FIG. 7 A pedigree (for key see Fig. 1) of limb-girdle muscular dystrophy suggesting autosomal recessive inheritance (from Walton, 1956a)

in the sibships of the propositi approached the 1 3 ratio to be expected with an autosomal recessive gene. If sibships containing less than three members were excluded, the ratio of 15 48 was even nearer the expected incidence.

In one of Walton's (1955) families, two affected individuals had an affected parent, an observation which would at first sight raise the possibility of dominant inheritance, but it is of course conceivable that the affected parent had married a heterozygous carrier of the gene Basing his calculation upon the incidence of the disease in Northeast England, Walton (1956a) calculated the gene frequency (q) to be [illegible] Hence an affected individual would be expected to produce affected children once in every 316 marriages. In another family (Fig 8) two sufferers had an affected niece As each sib of an affected individual has a 1 in 2 chance of being a heterozygous carrier and a 1 in 316 chance of marrying an individual of like genotype, the chance of an affected individual having an affected nephew or niece, if there is no consanguinity is 1 in 632 If random mating can be assumed in the population and the case frequency (^{2}) is 1/100 000 as it was in North east England, then the carrier frequency would be 1/158 and two carriers would be expected to mate and to produce affected children once

in every 25 000 marriages. As so few families have been reported showing cases in previous generations, there is no evidence to suggest that anticipation occurs in this form of muscular dystrophy but penetrance appears to be complete.

Studies carried out by Stevenson *et al* (1955) in an attempt to demonstrate linkage between the gene for this condition and other identifiable genes, have not as yet been fruitful

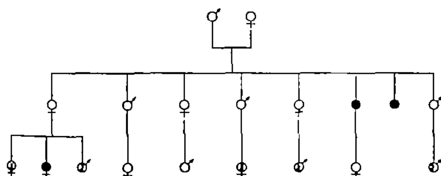


FIG. 8. A pedigree (for key see Fig 1) of limb-girdle muscular dystrophy in which two affected individuals had an affected niece (from Walton, 1956a)

4 *Distal Myopathy*

In 1902 Gowers lectured at the National Hospital upon a type of muscular dystrophy which began in the periphery of the limbs. Since then, doubt has been cast upon the existence of a distinctive distal form of myopathy (Critchley 1949) However Welander (1951) basing her account upon a personal examination of 249 patients, has confirmed that a distal form of the disease does indeed occur and is not rare in Sweden. The disease is characteristically benign begins in the small muscles of the hands and in the feet and legs between the ages of 40 and 60 and occurs in both sexes, though more men than women are affected. Characteristically however the disease is inherited as an autosomal dominant character though possibly with some sex limitation (to males)

5 *Barnes Type Myopathy*

In 1932 Barnes described a form of muscular dystrophy apparently unique, which was inherited as an autosomal dominant character of complete penetrance in a single family In the early stage, the condition

typically gave true muscular hypertrophy with increased strength this was followed by a pseudohypertrophic stage and an atrophic stage and the condition terminated in a severe atrophic myopathy of distal distribution. It may be that this disorder was a unique clinical and genetic entity and it is difficult in retrospect to draw any firm conclusions concerning the nosological status of this disease. Nevertheless, despite the fact that Barnes (1932) observed myotonia in only one of his cases, it must be admitted that in many respects the course of events in his patients resembled that seen in some families of dystrophia myotonica (to be considered below)

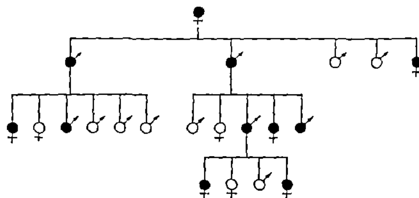


FIG. 9. A pedigree (for key see Fig 1) of progressive ophthalmoplegia (probably ocular myopathy) (redrawn from Beaumont, 1900)

6 *Ocular Myopathy*

In cases of muscular dystrophy affecting the external ocular muscles, the condition begins with ptosis or with diplopia and progresses to complete bilateral ophthalmoplegia. In most cases, the upper facial muscles are weak and there may also be dysphagia and relatively slight weakness and atrophy of neck, trunk, and limb muscles in a proportion of cases (Kiloh and Nevin, 1951) Many such cases have been previously referred to as examples of progressive nuclear ophthalmoplegia, but pathological observations have revealed that in most cases the disorder is myopathic. About 50% of reported cases have been isolated but when the condition is inherited it appears generally to be due to an autosomal dominant gene (Fig 9) in one pedigree, the condition was transmitted through four generations, in both sexes, without a break (Beaumont, 1900) The modern tendency to assume that all such cases

are myopathic, may however, be inaccurate I have seen a case of total bilateral ophthalmoplegia in a child who also showed retinitis pigmentosa, deafness, cerebellar ataxia, and a spastic paraplegia, and it seems possible that in this case, as in other examples reported by Walsh (1947) the pathological changes responsible for the ocular disturbance were in the oculomotor nuclei However, pathological studies in a case of this type (Stephens, Hoover and Denst, 1958) have revealed definite myopathic charges in the ocular muscles. Much more information must be collected before the genetic background of ocular myopathy can be accurately defined although, as Kiloh and Nevin (1951) point out, the condition shows certain resemblances to facio-scapulo-humeral dystrophy for the present it must be regarded as a distinctive entity

7 *Conclusions*

It may be concluded that the uncommon distal and ocular forms of muscular dystrophy and possibly the Barnes type must be regarded as distinctive forms of the disease and that they are often produced by autosomal dominant genes. Most of the commonly occurring cases of muscular dystrophy however can be classified in three groups, the Duchenne, facio-scapulo-humeral and limb-girdle types. The Duchenne type is usually inherited as a sex linked recessive character the facio-scapulo-humeral as an autosomal dominant (possibly with incomplete penetrance or sex limitation in certain families) and the limb-girdle as an autosomal recessive.

B. The Myotonic Syndrome

The phenomenon of myotonia consists in a failure of voluntary muscle to relax immediately when voluntary innervation ceases (Martin 1947) characteristically it is seen best in clinical practice as an inability to release the grip while tapping the belly of a muscle may result in the formation of a "dimple" which can persist for a few seconds. Myotonia is clearly due to an abnormality in the muscle fiber itself and not in the motor nerve or end plate, as it persists after section or blocking of the motor nerve and after curarization (Brown and Harvey 1939 Denny Brown and Nevin, 1941)

Myotonia is a feature of four principal clinical syndromes, namely myotonia congenita, dystrophia myotonica, paramyotonia, and symptomatic myotonia or myotonia acquisita. In myotonia congenita, as

described by Thomsen (1876) in members of his own family, the disease is typically present from birth and is characterized by severe stiffness and difficulty in relaxation of the entire voluntary musculature the stiffness is accentuated by cold and relieved by exercise, while generalized muscular hypertrophy is common. The condition is benign and tends to improve throughout life. When the myotonia, instead of being relieved by activity is exaggerated thereby this is known as myotonia paradoxa. Dystrophia myotonica (Steinert, 1909, Batten and Gibb 1909) by contrast, begins usually in adult life and is characterized by *myotonia which may be localized to the small hand muscles, forearms,* and tongue and by cataract, frontal baldness (in the male) gonadal atrophy mental deterioration, facial myopathy sternomastoid weakness, and a progressive myopathy of peripheral distribution in the limbs. Cardiac changes and mental deterioration are also common as is an increased thickness of the vault of the skull and a small sella turcica (Caughey 1952 Walton and Warrick, 1954) while some patients have hyperostosis frontalis interna (Jequier 1950) Paramyotonia (Eulenburg 1886) is similar to myotonia congenita in that the condition is congenital and generalized throughout the musculature, but in this condition, myotonia appears only on exposure to cold and is then followed by severe generalized weakness. Each of these conditions appears to be genetically determined and to be inherited by an autosomal dominant mechanism. Myotonia acquisita, on the other hand, has been described as an acquired disorder often beginning in adult life (Talma, 1892 Krabbe, 1934) and appearing sometimes in localized form, as a manifestation of various neurological and endocrine disorders Much controversy has arisen and persists to the present day concerning the interrelationship of these conditions, but consideration of this vexed question may conveniently be deferred until after the consideration of their genetic characteristics.

I *Myotonia Congenita*

The pedigree of Thomsen s family was brought up to date by his *great nephew Nissen, in 1923 (Fig 10) In this family the manifes*tations of the disease were relatively constant throughout the generations and the condition was clearly due to an autosomal dominant gene showing complete penetrance. A similar family has been reported by Birt (1908) Bell (1947) reviewed 21 reported families, containing 100 *individuals, of which the largest was Thomsen s own with 39 affected*

members. She pointed to the inconstant occurrence of hereditary psychosis in some families, including Thomsen's, but there were no other associated defects of note, and the disease did not appear to shorten life significantly. Thomasen (1948) described 4 personal families, in 2 of which the disease appeared to be transmitted as an autosomal dominant character with incomplete penetrance, while in each of the other 2, the disease was present in only a single sibship suggesting either recessive inheritance (there was consanguinity in 1 family) or a mutation.

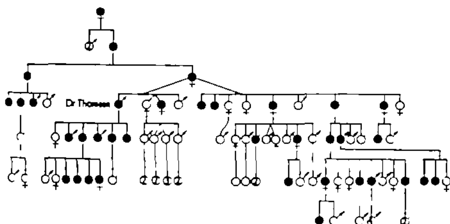


FIG. 10. The inheritance of myotonia congenita in Dr. Thomsen's own family (redrawn from Thomasen, 1948). For key to pedigree see Fig. 1.

2. *Dystrophia Myotonica*

A striking feature of this disorder is the remarkable variability of the clinical manifestations, a variability which may be apparent not only in any comparison of different families, but also between members of the same family. It is relatively common to find that cataract alone, or combined with minimal dystrophic features of late onset, may have been present in one generation, while in the next, the condition appears in a relatively severe form in early adult life (Greenfield, 1911). Sometimes myotonia is severe and generalized, and is the main cause of disability for many years before dystrophic features develop (Denny Brown and Nevin, 1941; Maas and Paterson, 1950; Lynas, 1957), while in other instances clinical myotonia is never demonstrable at any stage (Fearnsides, 1915). Furthermore, Maas and Paterson (1939, 1943, 1950) have pointed to the frequency with which a single

family may contain members who are severely affected by the disease and others who show only slight manifestations. For this reason, they stressed the importance of careful examination of all available relatives.

Despite the remarkable variability in clinical presentation of this disease, there is now general agreement that it can be attributed to a autosomal dominant gene (Bell, 1947 Thomasen, 1948 de Jong 1955, Lynas, 1957) although Maas and Paterson (1943) suggested that possibly the individual features of the disease, such as cataract, myotonia, and myopathy were due to separate genes which were recessive in isolation but dominant in combination. However the latter

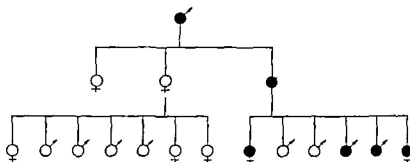


FIG. 11 A pedigree (for key see Fig. 1) of dystrophia myotonica (previously unpublished) illustrating simple dominant inheritance.

hypothesis would appear to be invalidated by the fact that the authors found no example of "skipping" of a generation in their own material Bell (1947) also found some difficulty in accepting that simple dominant inheritance was invariable in view of the fact that in many of the families which she reviewed the disease was passed on by individuals who were apparently unaffected. However the fallacies inherent in any large review of published material may have been responsible for this and the other inconsistencies which she mentioned, although it is apparent from the work of others (Lynas, 1957) that the penetrance of the gene is sometimes incomplete

Nevertheless, in a great many families, there is no evidence of "skipping" of generations and there seems to be complete penetrance (Figs. 11 and 12) Thus Thomasen (1948) in a personal review of 21 families containing 149 probable cases of dystrophia myotonica and 131 patients with cataract alone, found that, using Weinberg's proband method, the incidence of affected individuals in all the sibships studied was 46 ± 3 6% In the cases of de Jong (1955) which were discovered

in 19 sibships of 11 families the ratio of affected to unaffected individuals, if the propositi were excluded, was 24 22, or almost exactly the expected 1 1 ratio. Lynas (1957) who studied 55 cases in 13 families in Northern Ireland, and found the gene frequency to be 1.203 per 1 000 of the population discovered 42 affected and 67 unaffected individuals in the relevant sibships if the 18 propositi were excluded the ratio 24 67 was much less than the expected 1 1 but these sibships contained many children who might yet develop the disease. On the other hand 4 of her families contained cases in only 1 generation, suggesting incomplete penetrance of the gene or else a

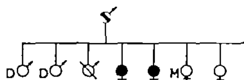


FIG. 12. A pedigree (for key see Fig 1) of dystrophia myotonica (previously unpublished) Note that the father had cataract alone note also the mental defect and high mortality in apparently unaffected sibs.

mutation She calculated that the figure 8 × 10⁻ gave a crude estimate of the mutation rate in Northern Ireland By contrast, Maas and Paterson (1943) found that if they included individuals with "suspicious" features suggesting minimal affection, the affected unaffected ratio in their series of cases exceeded 1 1

Among other features observed by Maas and Paterson (1943) in their families was clear-cut anticipation or a progressively early onset of the disease, with increasing severity in successive generations. They also noted that this process was often accompanied by a progressive decline of the family in the social scale and by diminished fertility Furthermore, fraternal anticipation was common, in that severe manifestations such as mental defect, were particularly common and the disease often began earlier in younger members of a sibship It was also apparent that in the unaffected sibs of patients with dystrophia myotonica there was an excessive infantile mortality as well as an over all mortality rate in early adult life which greatly exceeded that observed in the general population (Bell, 1947) Other workers have not noted any decline in social status (Lynas, 1957) and comparisons of the age of onset in sib pairs has revealed no evidence of fraternal anticipation (Penrose 1947 Thomasen, 1948 Lynas, 1957) Nor is

reduced fertility invariable (Thomasen, 1948) though it was noted by Lynas (1957) Apparent anticipation and an increased mortality in unaffected sibs are, however much more constant findings (Bell, 1947 Thomasen, 1948 Lynas, 1957) though as Penrose (1947) points out, selection of cases of simultaneous onset in parent and child may exaggerate the impression of antedating While agreeing that no disease shows the phenomenon more strikingly than dystrophia myotonica, he suggests that it is partly an artifact due to the exceptionally low correlation for age of onset in parent and child which is characteristic of this disease. Lynas (1957) found that if an onset with cataract was included, there was a high correlation for age of onset in parent-sib pairs in her series, but excluding the cases beginning with cataract, there was a zero correlation

In attempting to explain anticipation in these families, as well as the very variable manifestations of the disease, Penrose (1947) suggested that it is simplest to assume that the variations are due to the modifying affect of an allele upon a dominant gene. For instance, the parent with a late onset and a relatively mild form of the disease may have a "favorable" modifier which he cannot pass on to his affected children who are therefore more severely affected. In turn, these children with an "unfavorable" modifier may pass this on to their unaffected children and this "unfavorable" gene may be responsible for the "suspicious" features noted by Maas and Paterson (1943) and for the high mortality in unaffected sibs.

3 *Paramyotonia*

Since Eulenburg's (1886) original report, large families containing cases of paramyotonia have been reported by Stephens (1953) and de Jong (1955) In these and other reported families, the condition was clearly inherited as an autosomal dominant trait with complete penetrance (Fig 13) In Stephens family there was no example of transmission of the disease by an unaffected individual and the relevant sibships contained 61 affected and 60 unaffected persons.

4 *The Relationship between Myotonia Congenita, Dystrophia Myotonica Paramyotonia, and Myotonia Acquisita*

Much controversy persists as to whether these conditions are distinctive diseases or variants of a single disease process. Grinker (1943) Klingler (1948) and Maas and Paterson (1950) believe in the essential

unity of myotonia congenita, dystrophia myotonica, and paramyotonia, while Bell (1947) Thomasen (1948) and de Jong (1955) among others, consider that they are separate diseases, a view which is shared by many geneticists (Gates, 1946 Penrose, 1947 Roberts, 1948) It is true that the benign natural history of typical Thomsen's disease and the longevity observed in many affected individuals is in striking contrast to the usual course of dystrophia myotonica, which generally causes progressive disablement and death in middle life. On the other hand, many families have been reported which appear to illustrate a transition between the two disorders (Boeters, 1935 Maas and Pater

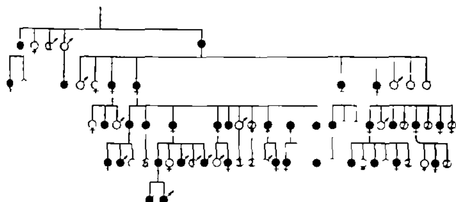


FIG. 13. A pedigree (for key see Fig 1) of paramyotonia showing dominant inheritance (family U redrawn from de Jong 1955)

son, 1950 Walton and Nattrass, 1954 Lynas, 1957) Not uncommonly in certain families generalized myotonia is the outstanding feature of the condition for many years, but dystrophic features develop subsequently although they remain minimal. It may be suggested that examples of this nature merely illustrate the extreme variability in clinical course of dystrophia myotonica, but if all cases developing minor dystrophic features are to be considered to be examples of this disease, then it is very difficult to find cases satisfying the criterion for diagnosing myotonia congenita, namely that myotonia is the only symptom or sign throughout life. In the case of paramyotonia, there is much less reason for distinguishing the condition from myotonia congenita, as myotonia in all forms is invariably made worse by cold The principal distinguishing feature of this complaint, namely the paralysis which follows cold has clearly been due to the fact, in some families at least, that myotonia and muscular stiffness has become so

extreme on exposure to cold that the patients were unable to move. Furthermore, in a recent report (French and Kilpatrick, 1957) of a family showing dominant inheritance through four generations, one affected individual showed clearly defined features of dystrophia myotonica. Myotonia paradoxa, too can hardly be regarded as an independent entity as the myotonia has increased following exercise, rather than decreasing as is more usual in several reported families of Thomsen's disease, and in paramyotonia (de Jong, 1955) There are good reasons for suggesting that the condition referred to as hypertrophia musculorum vera (Friedreich, 1863 Spiller 1907 Maxwell 1947) is in reality a further variant of the myotonic syndrome and the same may well have been true of the Barnes type of muscular dystrophy (Barnes, 1932) Cases of true myotonia following exercise should probably be distinguished from others in which progressive muscular aching and stiffness, without demonstrable myotonia, follow exertion (Marshall, 1952) in such individuals, a defect in the utilization of muscle glycogen may sometimes be demonstrated (McArdle, 1951)

From the genetic point of view myotonia congenita, dystrophia myotonica, and paramyotonia all show dominant inheritance. Walton and Nattrass (1954) have suggested that although the three conditions may be regarded as distinctive clinical syndromes, they probably represent variations of a single disease process. Whether they are due to the same gene, with different degrees of modification of its effect in different families, or to three separate genes, cannot be decided upon the evidence at present available.

So far as myotonia acquisita is concerned the existence of this condition as a specific entity must also be called into question. In Thomasen's (1948) view many cases so diagnosed were in fact examples of myotonia congenita or dystrophia myotonica. It is also true that a myotonoid disturbance of skeletal muscle (Hoffmann 1897) or generalized muscular hypertrophy (Debré and Semelaigne, 1935) may occur in myxedema. Clinically and electromyographically however the abnormally slow muscular contraction and relaxation which are seen in this condition are different from true myotonia. The same is probably true of the symptomatic "myotonia" which is occasionally seen in a variety of neurological conditions, including polyneuritis (Wortter Drought and Sargent, 1952) progressive muscular atrophy (Cabot Case 40442 1954) and polymyositis (Layani *et al.*, 1955) Probably this phenomenon is better referred to as pseudomyotonia, rather than

myotonia acquisita, in order to distinguish it from the inherited disorder

5 *Conclusions*

It may be concluded that myotonia congenita (and its variant myotonia paradoxa), paramyotonia, and dystrophia myotonica are all inherited as autosomal dominant characteristics, though the manifestations of dystrophia myotonica particularly may be modified giving rise to apparent incomplete penetrance or anticipation in some families this modification may be due to the allelic gene It is not yet certain whether the three conditions are independent entities or variants of a single disease process, nor can it be decided whether they can be attributed to the variable effects of a single gene or to three different genes.

C. Familial Periodic Paralysis

In familial periodic paralysis, repeated attacks of paralysis of the skeletal musculature occur which can occasionally be severe enough to cause death (Holtzapple, 1905) but more often spare the respiratory and pharyngeal muscles. The disease may begin in the first year of life (Buzzard, 1901) or at any age, and the attacks, while they are characteristically present on waking in the morning and may be induced by exercise or by a large carbohydrate meal, vary in duration from a few hours to a few days (Talbott, 1941) Occasionally the weakness is localized to the muscles of a single limb (Ziegler 1949) In patients who have had numerous attacks permanent muscular weakness and wasting may ensue (Biemond and Daniels, 1934 Bickerstaff, 1953) while pseudohypertrophy has been reported (Tyler 1950)

Aitken *et al* (1937) showed that attacks were often associated with a fall in the serum potassium and could be relieved by potassium, but several families have been described in which there was no fall in serum potassium during attacks (Tyler *et al.* 1951 McArdle 1956) and sporadic cases can occur in which the level is actually raised when weakness is at its height (Bull *et al.* 1953) Furthermore, the condition may occur in combination with thyrotoxicosis (Dunlap and Kepler 1931 Robertson, 1954) Conn *et al.* (1957) found that the attacks could be related to periods of sodium retention, due to intermittent secretion of aldosterone. It seems evident that the condition is not a specific disease but a syndrome resulting from a metabolic disorder

passage of myoglobin in the urine, are not uncommon in individuals who show no other evidence of muscular disease, though permanent muscular wasting may sometimes develop after repeated attacks (Meyer Betz, 1911) Hed (1955) described 3 brothers, each of whom had suffered repeated attacks of this nature, but most of the reported cases have been sporadic (Reiner *et al.* 1956) The idiopathic paroxysmal form of myoglobinuria seems likely to be due to an intermittent disorder of muscle metabolism and Hed's (1955) experience suggests that this may sometimes be genetically determined.

G Other Muscle Diseases

1 *Myasthenia Gravis*

The most characteristic feature of myasthenia gravis is an abnormal degree of fatigability of skeletal muscle, and this fatigability may be partially or completely reversed by anticholinesterase drugs. The disease is much more common in women than in men, and most cases are sporadic. A transient myasthenic syndrome can occur in babies of myasthenic mothers presumably due to transmission of a cholinesterase like substance across the placenta, while congenital myasthenia gravis has also been described (Schlezinger and Yaskin, 1954) in children of apparently normal mothers. This condition too invariably remits.

Myasthenia gravis occurring in more than one sib has been reported by Bing (1939) Hart (1927) Riley and Frocht (1943) and Mancini Ungaro (1945) and Noyes (1930) described its occurrence in a father and two daughters Rothbart (1937) reported three brothers in whom myasthenia gravis began between 6 weeks and 6 months of age, while Levin (1949) described congenital myasthenia in two sibs. It is now becoming apparent that this condition is probably a syndrome of varied etiology and not a distinctive disease (Rowland, 1955 Paterson, 1956) Whatever the nature of the biochemical lesion responsible for the condition, it is apparent that this may sometimes afflict more than one member of a family though no distinctive genetic pattern emerges.

2 *Amyotonia Congenita*

It is apparent (Brandt, 1950 Walton, 1956b) that most cases of severe generalized hypotonia of the skeletal muscles, present at birth, which many would call amyotonia congenita (Oppenheim 1900) are suffering from spinal muscular atrophy of the Werdnig Hoffmann

type, a disorder which will be considered below In a few instances this clinical picture may result from primary glycogen storage disease of the muscles (Günther 1939 Clement and Godman, 1950) the occurrence of this condition in 2 sibs has been reported by Krivit *et al* (1953) Glycogen storage disease of the hepatic form is probably inherited as an autosomal recessive character (Hanhart, 1946 Klein, 1953) but the muscular form is biochemically different and its mode of inheritance has not yet been established (Krivit *et al.* 1953)

In addition to these disorders it is apparent that some infants with congenital hypotonia are suffering from a benign disorder (Brandt, 1950) Walton (1957) reported 17 such cases under the title of "benign congenital hypotonia" 9 recovered completely but 8 had small, weak muscles throughout life. All of these cases were sporadic. However, the cases with incomplete recovery were strikingly similar to the "benign congenital myopathy" which Turner (1940 1949) described in 6 of 13 members of a sibship and to the "congenital universal muscular hypoplasia" of Krabbe (1946) which Ford (1952) has described in a mother and daughter Shy and Magee (1956) have described a similar disorder affecting 5 patients in 3 generations of a family the pattern of inheritance suggested a dominant gene with incomplete penetrance. In this family histochemical studies of muscle biopsy specimens revealed a curious abnormality of the central portion of many muscle fibers. Until more is learned about the nature of these conditions giving rise to benign congenital hypotonia, it would be unwise to draw any conclusions concerning their etiology and inheritance. It is apparent, however that they can be distinguished from congenital laxity of the ligaments (Finkelstein, 1916 Sutro 1947) a condition which may be inherited as an autosomal dominant characteristic and in which there is a generalized hypermobility of joints (as in contortionists) but no muscular weakness.

IV NEUROMUSCULAR DISEASES

A. MOTOR NEURON DISEASE

The term motor neuron disease is used in Great Britain to identify that progressive process of degeneration of the upper and lower motor neurons which may present in three clinical forms, namely progressive muscular atrophy progressive bulbar palsy and amyotrophic lateral sclerosis. In the United States, the condition is often called motor sys-

tem disease or else the term amyotrophic lateral sclerosis may be used to identify the condition in all its clinical forms (Kurland and Mulder 1954) In progressive muscular atrophy wasting, weakness, and often fasciculation of peripheral limb muscles dominate the clinical picture, while in progressive bulbar palsy the pharyngeal and laryngeal muscles and the tongue are first affected and there may be signs of bilateral corticospinal tract lesions. In amyotrophic lateral sclerosis, weakness and spasticity of the limbs due to corticospinal tract dysfunction predominate, while there may be minimal muscular atrophy and fasciculation around the shoulder girdles. However all combinations of the

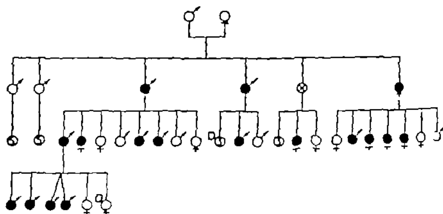


FIG. 15. The inheritance of progressive muscular atrophy as an autosomal dominant characteristic in the Farr family of Vermont (redrawn from Osler 1880 Brown, 1951) For key to pedigree see Fig 1

three clinical forms occur In most cases, the onset of the disease is between the ages of 35 and 55 years many affected individuals die within 2 years of the onset, but some may survive for 10 years (Müller 1952) or even, exceptionally for 35 years (Lawyer and Netsky 1953)

Most cases of this disease are sporadic Wechsler *et al.* (1944) found no evidence of an inherited factor in 81 cases, van Bogaert (1949) in 67 cases, and Müller (1952) in 190 cases. However Osler (1880) described 13 cases occurring in 1 family in Vermont and the pedigree of this family (Fig 15) has recently been extended by Brown (1951) In the late nineteenth century at least 18 extensive pedigrees containing 90 affected individuals were described and Kurland and Mulder (1955) have recently reported 6 personal families with 34 affected individuals, while Robertson (1953) has reported progressive bulbar palsy showing

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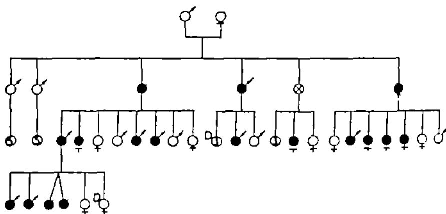


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dominant inheritance in a single family The families reported by Myrianthopoulos and Brown (1954) contained many cases of progressive ophthalmoplegia which may have been myopathic. In virtually all of the reported families, which were of many different races, the pattern of inheritance suggested an autosomal dominant factor sometimes with incomplete penetrance.

Of great interest are the large number of cases observed in the Chamorro people on the island of Guam (Arnold *et al.*, 1953 Koerner, 1952, Kurland and Mulder 1954) On this island the prevalence rate of the disease is about 200 per 100 000 population, or 50 times as great as in other parts of the world and the condition is responsible for 8–10% of adult deaths (Kurland and Mulder 1954) The clinical picture in these cases appears to be identical with that observed in sporadic cases elsewhere and yet on Guam the condition appears to be due to an autosomal dominant gene, though sometimes with incomplete penetrance and with expression more frequently in the male than in the female.

It is not yet certain whether the disease process in the hereditary cases is different from that in the commoner sporadic form however it is apparent that motor neuron disease of typical clinical presentation can be genetically determined So many sporadic cases occur that it seems inconceivable that they could all be due to mutation indeed in these individuals there is no evidence that inherited factors play any part in the etiology of the disease.

B Peroneal Muscular Atrophy

Peroneal muscular atrophy (Charcot Marie Tooth) is one of the most benign of all chronic neurological diseases. It gives rise to a characteristic slowly progressive weakness and wasting of the peripheral lower limb muscles, but the atrophic process stops short in the lower thighs to give the typical "inverted champagne bottle" appearance. In most cases, the small muscles of the hands and the forearms are eventually affected, while variable sensory loss, usually slight and of "posterior column" type may be observed in the periphery of the limbs. Although it was initially believed that this was a disease of the spinal cord (Charcot and Marie, 1886) Tooth s (1886) concept of the condition as a form of chronic peripheral neuropathy is almost certainly correct (England and Denny Brown, 1952) Despite the severity of the muscular wasting most affected individuals remain active until

late in life despite complete bilateral foot drop and claw hands, and remarkable longevity in affected individuals is not uncommon (England and Denny Brown, 1952 Hierons, 1956)

Bell (1935) in an extensive survey of published cases, showed that in a relatively small group of families, there was clear evidence of transmission of the disease as a sex linked recessive character (Herringham, 1889 Church, 1906) In a further small group of families, the disease had occurred in only 1 sibship and in these an autosomal recessive gene seemed probably to be responsible, though it was impossible to exclude sex-linkage in the families containing only affected

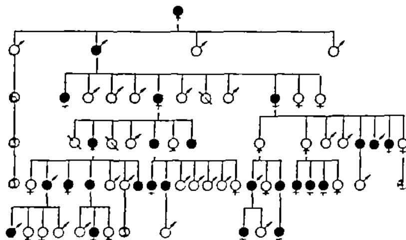


FIG. 16. A pedigree (for key see Fig 1) of peroneal muscular atrophy indicating autosomal dominant inheritance with occasional incomplete penetrance (redrawn from Hierons, 1956)

males, or incomplete penetrance of a dominant gene in the others. In the remaining and largest group of families, which included 429 affected individuals, i.e. 69% of the 620 published cases, there was clear evidence of autosomal dominant inheritance, with no clear evidence of sex limitation, as 225 males and 204 females were affected. In certain families, there appeared to be incomplete penetrance, as the disease was transmitted by an unaffected parent, but it must be remembered that some individuals might have been minimally affected with a forme fruste of the disease, while other parents might have died before the disease become manifest. Recent reports have confirmed that dominant inheritance of this condition is usual (Fig 16) In the family of England and Denny Brown (1952) the disease was passed through

7 generations without a break but the gene did not show all the characteristics of complete penetrance as the number of unaffected individuals generally exceeded the affected in each generation. In another large family reported by Hierons (1956) many of the members, both affected and unaffected achieved distinction in public life and records are available covering 8 generations. Again the pattern suggested autosomal dominant transmission but this time there was more evidence of incomplete penetrance, with occasional "skipping" of an individual who transmitted the disease.

The suggestion made by Allan (1939) that the recessive form of peroneal atrophy is the most severe was not borne out in Bell's (1935) analysis. There was some suggestion of anticipation in the family reported by Schwartz (1944) but again this is exceptional indeed Haldane (1941) found a remarkably constant age of onset in different generations of a single family England and Denny Brown (1952) noted that in their family severe cases began early mild or "forme fruste" cases late, but that examples of each were apparently scattered at random throughout the generations

Attempts to demonstrate linkage between the gene for peroneal atrophy and those for tasting phenylthiocarbamide (Bell, 1935) and for the blood groups (Hierons, 1956) have so far been unsuccessful

It may be concluded that peroneal muscular atrophy is generally the result of an autosomal dominant gene, often showing incomplete penetrance, but that examples of sex linked and possible autosomal recessive inheritance of the disease are on record. The relationship between this condition and other members of the hereditary ataxia group will be considered shortly

C. Progressive Hypertrophic Polyneuritis

In this progressive form of peripheral neuropathy first described by Dejerine and Sottas (1893) the distribution of the muscular weakness and wasting is very similar to that observed in peroneal muscular atrophy, but in addition there is a striking palpable hypertrophy of peripheral nerve trunks, shown histologically to be due to an "onion skin" hypertrophy of the sheath of Schwann. Sensory loss in the periphery of the limbs is usual but it is rarely obtrusive. Pupillary abnormalities, such as anisocoria and a slow light reflex, are common (François and Descamps, 1949) Among others Russell and Garland (1930) Sloane (1939) Thévenard *et al* (1956) and Bedford and James

(1956) have described families containing affected individuals. Snyder (1941) suggested that the condition is usually due to a recessive gene, as many sporadic and isolated cases occur However in the families reported by Russell and Garland (1930) and by Bedford and James (1956) the pattern of inheritance was typical of autosomal dominance (Fig 17) Bedford and James (1956) pointed out that initially they believed that their family contained only two affected sibs, but careful examination of relatives subsequently indicated that this impression was false.

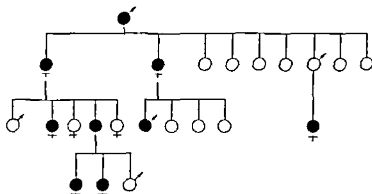


FIG. 17 A pedigree (for key see Fig. 1) of interstitial hypertrophic polyneuritis, indicating dominant inheritance with possible incomplete penetrance (redrawn from Russell and Garland, 1930)

D PERONEAL ATROPHY HYPERTROPHIC NEURITIS, AND THE OTHER HEREDITARY ATAXIAS

Although in some families, peroneal atrophy and hypertrophic neuritis may "breed true" in many affected individuals, these diseases may be found in combination with other member diseases of the "hereditary ataxia" group including particularly Friedreich's ataxia and optic atrophy Thus Spillane (1940) Roth (1948) Shepherd (1955) and Hierons (1956) among others, have reported families in which some affected individuals showed signs not only of peroneal atrophy, but also of Friedreich's disease. Retinitis pigmentosa was observed in 2 individuals with hypertrophic neuritis in the family of Bedford and James (1956) while severe sensory changes like those of hereditary sensory radicular neuropathy have been reported in peroneal atrophy (England and Denny Brown, 1952) Biemond (1928) found hypertrophy of peripheral nerves in only 1 of several affected

members of a family in which the manifestations were otherwise characteristic of peroneal atrophy The heredopathia atactica polyneuritiformis of Refsum (1946) and the familial areflexia (with pes cavus and dysarthria) of Roussy and Lévy (1926), as well as hereditary spastic paraplegia, which is sometimes accompanied by optic atrophy (Bickerstaff 1950), are probably other members of the group

Although many of these disease entities occur virtually in "pure" form in certain families, so many transitional and intermediate cases occur with considerable individual variability even within a single family that there is some substance in Cobb's (1944) view that the entire group of disorders may be included under a single generic heading namely "familial system disease of the neuraxis." In view of this variability it will undoubtedly prove difficult to isolate the gene or genes responsible for any single disorder in the group Indeed Haldane (1941) has suggested that each of the principal clinical syndromes may be due to a combination of 3 or 4 genes. As a result of interaction between these genes and modifiers (possibly multiple alleles) striking variations in clinical pattern and age of onset may be seen.

E. Infantile Spinal Muscular Atrophy

According to classic descriptions (Werdnig 1891 Hoffmann, 1893) this condition begins usually in the second 6 months of life with weakness and hypotonia of trunk and proximal limb muscles. Progressive paralysis of all the skeletal musculature ensues and death results, often within a few months of the onset, from respiratory infection. A small proportion of affected children live, though grossly disabled, for a number of years. It has already been pointed out that many similar cases with an onset at birth which have previously been called amyotonia congenita, rightly belong to this group, as do some children with arthrogryphosis multiplex congenita, in whom the muscular weakness and wasting was due to a degeneration of anterior horn cells beginning in fetal life (Walton, 1957)

Schinkus (1934) reviewed published cases of the Werdnig Hoffmann disease and found that 25% of the sibs of proposti were affected by the disease. This calculation suggested that the disorder was due to an autosomal recessive gene, as did the observations of Hanhart (1945) who, in reporting 29 cases, found consanguinity in 6 of the 14 families concerned Concordance in identical twins has been described by

Forbus and Wolf (1930) Thums (1938), Thomas (1941) and Ford (1944)

Brandt, (1949 1950) found 112 cases of this type in Denmark and *calculated that approximately 1 new case occurred each year per* 1,000 000 of population. In his material, there were 57 affected boys and 55 girls in the 70 families. Two mothers had affected children by more than one man and consanguinity had occurred in 4 families (5.8%) whereas in a control group of 290 families, the incidence was

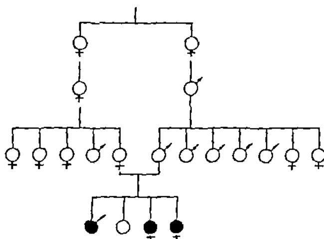


FIG. 18. A pedigree (for key see Fig 1) of infantile spinal muscular atrophy showing consanguinity and suggesting autosomal recessive inheritance (family XXIII redrawn from Brandt, 1950)

only 0 7% Expressivity of the gene was invariably complete using Weinberg's proband method, 30% of the sibs of propositi were affected. Despite the fact that this figure was considerably higher than that expected, *Brandt (1949 1950) concluded that the disease was due to an autosomal recessive character (Fig 18) though he made the reser* vation that incomplete dominance was a possibility in some families. In view of the fact that affected individuals never reproduce, the latter would seem unlikely and other recent workers (Arthurs 1954, Walton, 1956b) have agreed that the available evidence points strongly to autosomal recessivity though the unusually large proportion of affected individuals, particularly in some families, cannot yet be explained.

V GENERAL CONCLUSIONS

While it is apparent that much has been learned in the last 50 years concerning the inheritance of several important diseases of the muscular and neuromuscular systems, we know as yet very little concerning the means by which the responsible genes produce their effects upon the muscle (in muscle diseases) or upon its motor nerve supply (in neuromuscular diseases) Although much more information is needed concerning the inheritance of many of these disorders, and may be acquired through carefully planned field surveys, it is clear that the clinical geneticist must turn to his colleagues in biochemistry and histopathology for help in identifying the action of these deleterious genes. From such work stems the hope of finding some method of modifying their effects, and of eradicating these distressing diseases.

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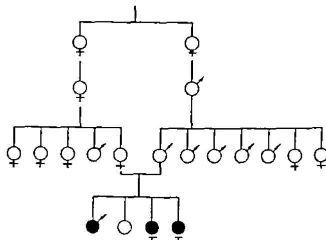


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CHAPTER XIII

Remarks on Muscle

ALBERT SZENT-GYÖRGYI

Most of the writer's thoughts, and both of his hands, are engaged in a different field of research only loosely connected with muscle. So if he cannot resist the temptation to follow the Editor's invitation to contribute to this book, he must limit himself to general remarks having no recent personal research experience to present.

Muscle, in his mind, is still a mystery—more than it ever was—because the accumulating observations remain isolated, or even seem to contradict one another.

The most basic observation about muscle chemistry was made a century ago by W. Kühne, who found that this tissue contained a great quantity of a specific protein which he called "myosin" which evidently played an important rôle in the specific function of muscle, contraction.

The next interesting development took place outside the muscle field. It was the gradual development of the knowledge of nucleotides, ATP and the high-energy phosphate bond "∼" which seemed to be the source of the energy which aliments various biological processes, including muscle contraction.

The first step toward a synthesis of these findings was made by Engelhardt and Ljubimowa (1939) who discovered that myosin could split off the terminal phosphate of ATP liberating thus the energy which it needed for its working.

Shortly afterwards actomyosin was discovered, its components actin and actin-free myosin, were isolated (Szent-Györgyi, 1945, 1947; von Ardenne and Weber (1941); Schramm and Weber, 1942). This protein complex showed, in a proper ionic atmosphere, violent changes in its physical state on addition of physiological concentrations of ATP. These changes involved a loss of charge and hydration and entailed a shortening of the fibrous protein particles. Threads, prepared from an actomyosin gel, which contained the fibrous protein particles in random orientation, simply shrunk on addition of ATP. However, if the particles had their axes arranged parallel to the axis of the thread, then on addition of ATP this latter shortened without getting thinner.

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maintained its volume, similarly to contracting muscle. The analogy between muscle contraction and the colloidal changes brought about by ATP in actomyosin could be brought even closer by the "glycerinated muscle" (Szent Györgyi, 1949) that is muscle fibers extracted with glycerol, which not only contracted on addition of ATP but developed the same tension as they developed maximally in vivo The conclusion seemed to be warranted that muscle contraction is an interaction of ATP actin, and myosin an interaction which entails violent changes in colloidal state and involves a shortening of the fibrous particles of the protein complex, actomyosin. The free energy which enabled the shortening fibers to lift a weight and do work is supplied by ATP the terminal ~ of which is hydrolyzed in the process. Thus far the observations seemed to converge toward a deeper understanding

Further development concerned the second part of the contraction cycle relaxation Marsh (1951) showed that muscle contained a factor which, in presence of ATP caused swelling of muscle fibers inducing changes analogous to relaxation. There are reasons to believe that this "Marsh factor" is actually responsible for the relaxation of muscle fibers. Since the actomyosin ATP system does external work and spends free energy in its contraction evidently its relaxed state is its high-energy the contracted its low-energy state [1] In a thermodynami

[1] It could be objected to that the relaxed state cannot be the high-energy state since relaxation is a passive process which demands no energy the contracted muscles being readily extended by the bones, after contraction is over The contradiction is only an apparent one. As contraction, that is, actual shortening, is but the secondary consequence of another reaction (see later) so also, the extensibility which follows contraction is but the secondary consequence of another primary reaction which preceded it. This primary process is probably no other than the dissociation of actomyosin into actin and myosin While actomyosin can have two states which differ by their energy content the extended and contracted one free myosin and actin have but one state, and hence the muscle can be extended practically without resistence after the dissociation of the protein complex has occurred. We have little information about the energetics of this primary reaction which makes the muscle extensible.

One could also object to the above thermodynamic outlook and make the theory that the contractile matter has no two states which differ in their energy content, being comparable to an elevator which can stop at any point and will move only if, and as long as, energy is supplied from an outside source. The situation between elevator and muscle however is different since energy to an elevator is supplied, usually by an outside source located far away The muscle machine has to include its own energy source and would thus be comparable to an elevator driven by an accumulator placed in the elevator itself Function, as in muscle, would have to involve loss of energy of the total system, and so the high-energy state would have to be the initial resting state the low-energy one the final stage after the work is done

cal sense muscle contraction is thus a spontaneous process and the contracted state is the more probable one. So, thermodynamically, the puzzle is not why muscle contracts, but how it can stay relaxed and what makes it relax, once contracted It seems likely that the muscle can stay relaxed because it contains no actomyosin, only actin and myosin, side by side, and so it also seems likely that what the Marsh factor does is to dissociate actin from myosin. The relaxing factor seems to help actomyosin to stay in this dissociated state because its inactivation, as can be effected by the addition of calcium, entails immediate contraction. How far it uses solely the ~ of ATP and how far other ~ s as that of creatine or carnosine phosphate, is unsettled.

ATP can thus be demonstrated to have two actions acting directly on actomyosin it produces contraction and acting through the relaxing factor it produces relaxation. It seems to be able to produce contraction only when the relaxing factor is inoperative. The triggering of contraction may thus be in a sense an inactivation of the relaxing influence.

The relaxing factor presents a number of most fascinating problems. Its function has been made still more enigmatic by the discovery of Kumagai *et al* (1955) according to which this factor can be spun down at relatively low speeds and thus can be located in granules as definite morphological entities This far all attempts have failed to show that a substance is produced by these particles which diffuses out of them and makes actomyosin relax. But, if nothing diffuses out, how can changes be induced in actomyosin by something which happens in (or on) microsomes? This is one of the most fascinating puzzles at present.[2]

Continuing the list of observations, bearing on the theory of muscle contraction, the studies should be mentioned which relate to the detailed structure of myosin (see Volume II, Chapter I) Myosin is not a homogeneous substance but is built of different segments Under the influence of proteolytic enzymes the myosin "molecule" breaks up into three different parts with widely different properties. Various enzymes produce, if not identical closely similar units, meromyosins, so that there are reasons to believe that these units were preformed even if the mechanism of the enzyme action, which liberates them, is still

[2] The Japanese workers find also a soluble fraction which cannot be centrifuged out. How far soluble enzymes, like phosphopherases (creatine carnosine or phosphopyruvic phosphopherase) complete the action of granules is under discussion at present.

under discussion. What is rather unexpected is the fact that only one of the three different meromyosins splits ATP and unites with actin while the other two have properties which suggest their participation in the process of shortening but neither split ATP nor form links with actin. But, if this is so and the "L-meromyosins" shorten and perform work and the "H meromyosins" liberate the energy, then how can the energy liberated by the H's do work in the L's?

In the extracted myosin the three meromyosin particles are attached to one another in series but there is a certain measure of incertitude about the distribution and interrelation of the meromyosins while they are in the muscle. What we have, for a long time, regarded as myosin with a molecular weight of about 10^6 g turned out to be a dimer formed out of two smaller molecules which associated only after their extraction (Laki and Carroll, 1955). But if this is so, then is it certain that what we regard as "myosin" today is not something formed out of meromyosins only in our flask? Does the muscle contain "myosin" at all and not only meromyosins? Recent immunological studies give support to this latter assumption suggesting that the three different meromyosins have a different distribution within the "A band. These are problems which have yet to find the final answer at present, they show the incertitude of our knowledge, which admits doubts even about the existence of myosin which, for almost a century was the basis of all of our ideas.

Even the nature of the meromyosins is doubtful. If treated with urea they fall to a great extent into "protomyosins," very small uniformal units of equal size of a molecular weight of somewhat less than 5000 g. Urea does not split covalent bonds, only H bonds. If we define a molecule as a structure built of atoms, held together by covalent bonds, then the meromyosins, and with it the myosin, are not molecules at all. What is remarkable is the fact that the myosin is not decomposed into protomyosins by urea, only meromyosins are decomposed by it. This means that the myosin particle has a "structure stability," a stability like that of an egg shell which is linked to the intactness of the whole. Once this is damaged the entire compound tends to go to pieces. Nature does not indulge in luxuries or superfluous complications and so all this must have a meaning and close bearing on the theory of contraction. In the writer's opinion we will understand muscle only when we are able to tell how all these very involved structural details bear on function, and what are their meaning and importance.

While these chemical studies directed the attention, more and more, toward the submolecular level H Huxley's magnificent electron microscopic pictures put histological structures into focus again (see Volume I, Chapters VI and VII) These pictures showed cross-striated muscle to contain two sorts of fibers, thick ones and thin ones The former occupied the A band while the thin ones reached from the Z lines to the H band. Earlier chemical studies support Huxley's contention that the thick threads were myosin (Hasselbach 1953), and no definite objection can be raised against his assumption that the thin ones were actin. According to the theory developed by H Huxley A. Huxley and J Hanson contraction consists of the thin filaments being pulled in between the thick ones, which process could account for about 30% shortening Since the body muscles, as a rule, shorten no more than 10–15% the theory can account for the whole dimensional changes under physiological conditions. The motion pictures taken by A. Huxley of living muscle can leave little doubt in the spectator's mind about the basic correctness of the theory

Undoubtedly the theory means a most important step in our understanding of the mechanics of muscle contraction. In a way it seems to be at variance with the earlier theories which supposed major changes in colloidal state and folding The Huxley theory of sliding filaments invokes only a sliding of the unchanged filaments. In the writer's opinion the Huxley theory is, by no means, contradictory only complimentary Evidently the sliding of the filaments is the result of some change which had to precede it, which changed the situation in such a way that the free energy of the system decreases when the thin filaments come to lie between the thick ones. What this primary change is, which is actually responsible for the transformation of chemical energy into mechanical work, we still do not know It is this primary process which declares itself in superprecipitation when it takes place in an actomyosin suspension, or declares itself in synaeresis or contraction if it occurs in an actomyosin thread. In the whole muscle it declares itself also in the development of the "active state" of Hill The sliding is

[1] The authors speak about the "sliding" of filaments and the theory is often called that of the "sliding filaments" In the writer's opinion this name is misleading or at least unjustified. "Sliding" involves the idea that the two kinds of filaments remain straight and just slide along one another as two sticks would. The British authors actually think that this is what happens but have no evidence for it. What happens to the thin filament when it is pulled in between the thick ones we do not know It seems possible, though not likely that it does fold up or becomes creased.

its secondary consequence. In cross-striated muscle which has to work fast, this sliding makes a rapid shortening of the whole system possible. In smooth muscle there is no analogous arrangement which could make a rapid sliding possible and hence the only secondary change we see is a shortening which must be due to some sort of folding, equally a secondary consequence of the primary change. This folding is a much slower process than the sliding. Thus folding and actual shortening of fibrous particles can occur equally in cross-striated muscle though not under physiological conditions. The sliding of filaments allows a shortening of 30% but cross-striated muscle can shorten by 80% Fifty per cent of this must be due to some change analogous to the shortening of smooth muscle

The actual mechanism of muscle contraction, the chemical reactions and energy changes which introduce it, and the physical changes which accompany it, are not the only blanks on our map There are morphological structures as the endoplasmic reticulum of Pallade and Porter (see Volume I Chapter VI), the function of which is entirely unknown, which may hold the key to important problems The other chapters on muscle function, as the triggering off of contraction by changes in the membrane, the communication between membrane and actomyosin are about equally obscure as the function of the contractile matter itself. Everything seems possible and even my "window field" (Bay *et al.*, 1953) which seemed to be dead for some time seems to get a new lease on life by recent studies (Csapo and Suzuki, 1958 Csapo, 1958)

Summing up we can say that the central happening of muscle contraction, in which chemical energy is translated into mechanical work, is just as much a mystery as it ever was and one might ask why in spite of the great number of interesting observations made, we still do not understand muscle Not only do we not understand it, the newer observations, instead of adding to our understanding, seem only to nibble away our old ideas. One may wonder why muscle research has bogged down and why this lack of understanding (Szent-Györgyi, 1945 1947) My impression is that our basic outlook on such complex biological reactions as muscle contraction is wrong Biochemistry and our whole biochemical thinking is dominated too much by classical chemical concepts. We make progress only in the analysis of structures and reactions which can be expressed by letters and dashes, the symbols of classical chemistry We make no progress in the analysis

of more subtle and more involved biological processes, as the production of mechanic, electric, or osmotic work. This may be for two reasons partly because these processes involve changes in the physical state of complex systems and are not the results of processes which could be duplicated in homogeneous solutions, and be expressed with letters and dashes. The other reason may be that we blinded ourselves by looking too much on classical chemistry and have failed to see that quantum mechanical changes may play a leading rôle with delocalized electrons and the like. The signal given by ATP in the electron spin resonance experiment pleads strongly for such an assumption (Isenberg and Szent-Györgyi in press) The writer's personal studies move in this direction and his preoccupation (Szent Györgyi 1945 1947) on this line may serve as an excuse for the grave shortcomings of this article.

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